PERIODICAL
#50
Denmark. Danish Biolog. Stat.
Report.
#5
LIBRARY
FISH & WILDLIFE SERVICE
PACIFIC OCEANIC FISHERY
INVESTIGATIONS

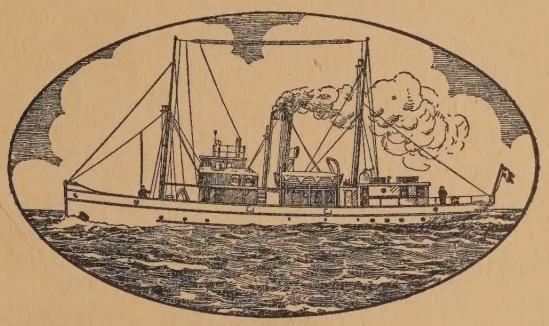
Report

of

The Danish Biological Station

to

The Ministry of Agriculture and Fisheries



L.

1947

By

H. Blegvad, Ph. D.
Director

Copenhagen. C. A. Reitzel.
1950.

The Reports from the Danish Biological Station (Nos. I—XXI) are published in the official Danish "Fiskeri-Beretning" for the years 1890—91 until 1912. After this year they appear as special publications. The Reports I—XXXII are edited by Dr. C. G. Joh. Petersen, the Reports XXXIII—XXXVI by Dr. A. C. Johansen and the later Reports by Dr. H. Blegvad.

I. *C. G. Joh. Petersen:* Fiskenes biologiske Forhold i Holbæk Fjord. (1890—91)*) 63 pp. 1 Chart. Out of print.

II. *Idem:* On the Eggs and Breeding of our Gobiidæ. (1891—92.) 9 pp. Two Plates. Out of print.

III. *Idem:* The Pelagic Life in Fænø Sound etc. (1892—93.) 38 pp. Tables. Out of print.

IV. *Idem:* On the Biology of our Flat-fishes and on the decrease of our Flat-fish Fisheries. (1893—94.) 146 pp. 2 Plates. 1 Chart. Many Tables. Out of print.

V. *Idem:* The common Eel (*Anguilla vulgaris* T.) gets a Particular Breeding-dress before its Emigration to the Sea. (1894—95.) 35 pp. With 2 Plates.
Etc. 64 pp. Out of print.

VI. *Idem:* The Yearly Immigration of young Plaice into the Limfjord etc. (1895—96). 48 pp. 1 Chart. 2 Tables. Out of print.

VII. *Idem:* Plankton-Studies in the Limfjord. 1897. 23 pp. 1 Map. 4 Tables. Out of print.

VIII. *Idem:* An Otter-Seine for the Exploration of Deeper-Seas. 1898. 24 pp. With 10 Figures. Out of print.

IX. *Idem:* Trawlings in the Skager Rack and the Northern Cattegat in 1897 and 98. 1899. 56 pp. With one Map. Out of print.

X. *Idem:* List of the "Aalerusestader" in Denmark etc. — Smaller Communications. 1899 and 1900. 37 pp.

XI. *Idem:* I. The Biology of the Cod in the Danish Seas. II. On other Codfishes in our Seas. III. Some General Observations on Protection, Legislation etc. IV. Alterations and Improvements on Otter-seines for Zoological Purposes. 1900 and 1901. 44 pp.

XII. *Idem:* I. Where, and under what Conditions, can the Eggs of Plaice be developed into Young Fish within the Skaw? II. On the Young Stages of the genus *Zeugopterus.* (With one Plate). III. Can we enter into Competition with the Foreign Steam-trawlers in our Seas outside the Danish Territorial Limit. 1902 and 1903. 33 pp.

XIII. *A. Otterstrøm:* Eggs and Young of Fishes in the Danish Waters. (Investigations during 1904 and earlier years.) 1903 and 1904. 85 pp.

XIV. I. *C. G. Joh. Petersen:* The Influence of Light on the Migrations of the Eel. II. *K. I. Gemzøe:* Age and Rate of Growth of the Eel. 1906. 39 pp.

XV. *Idem:* I. First Report on the Oysters and Oyster Fisheries in the Lim Fjord. (70 pp.) 1908.

XVI. *C. H. Ostenfeld:* On the Ecology and Distribution of the Grass Wrack (*Zostera marina*) in Danish Waters. With 9 figures. 1908. 62 pp.

XVII. *C. G. Joh. Petersen:* II. Second Report on the Oyster and Oyster Fisheries in the Lim Fjord. 1908. (24 pp).

XVIII. *C. G. Joh. Petersen:* I. The Yield of the Limfjord fisheries in recent years and the transplantation of Plaice in 1908. With 6 tables and 1 Chart. II. Biological investigations on the stock of Plaice in the Limfjord. With 3 Tables. III. The Yield of the Prawn fishery from 1885—1907 and its improvement by means of protection. With 3 Tables. 25 pp. 1909.

XIX. *Idem:* Some Experiments on the Possibility of combating the harmful Animals of the Fisheries, especially the Whelks in the Limfjord. 20 pp. 1911.

XX. *C. G. Joh. Petersen* og *P. Boysen Jensen:* Valuation of the Sea. I. Animal Life of the Sea-Bottom, its food and quantity. With 6 Tables, 3 Charts and 6 Plates. 81 pp. 1911.

XXI. *C. G. Joh. Petersen:* Valuation of the Sea. II. The Animal Communities of the Sea Bottom and their importance for marine Zoogeography. With 6 Plates, 3 Charts and an Appendix. 110 pp. 1913.

XXII. I. *P. Boysen Jensen:* Studies concerning the organic matter of the Sea Bottom. II. *H. Blegvad:* Food and Conditions of Nourishment among the Communities of invertebrate animals

*) The figures in ( ) refer to the "Fiskeri-Beretning" for the years quoted.

LIBRARY
FISH & WILDLIFE SERVICE
PACIFIC OCEANIC FISHERY
INVESTIGATIONS

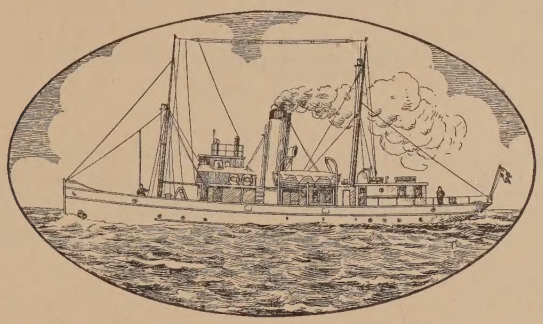
Report

of

The Danish Biological Station

to

The Ministry of Agriculture and Fisheries



L.

1947

By

H. Blegvad, Ph. D.
Director

Copenhagen. C. A. Reitzel.
1950.

CONTENTS

Page

Knud Larsen and Sigurd Olsen: Ochre Suffocation of Fish in the River Tim Aa. Drainage water from lignite pit as cause of the catastrophes at the Nørre Esp trout pond farm 1941—47 3

M. Christiansen and Aage J. C. Jensen: On a Recent and Frequently Occurring Tumor Disease in Eel 29

Ochre Suffocation of Fish in the River Tim Aa.

Drainage water from lignite pit as cause of the catastrophes at the Nørre Esp trout pond farm 1941–1947.

By

KNUD LARSEN and SIGURD OLSEN

CONTENTS

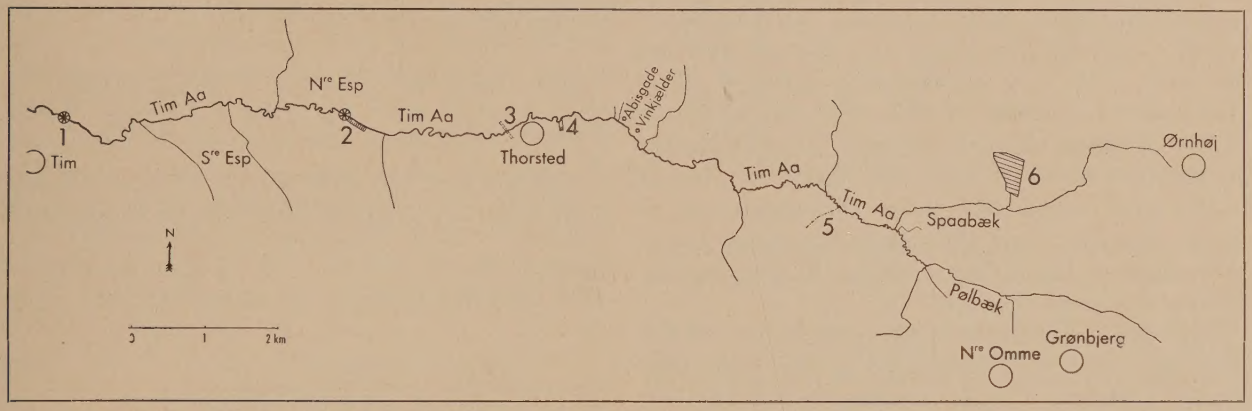
	Page
1. The river Tim Aa, situation and surroundings	5
2. The catastrophes at the pond farm and the investigations in 1943—44	5
3. Ochre suffocation and its causes	7
4. The free water fishery in the river Tim Aa 1942—45	9
5. The investigations in 1945—46, the end of the breaking of lignite in June 1946 and the catastrophe in January 1947	9
6. The analyses from April 1947	13
7. Principal data from the material analysed	18
8. Conclusion; termination of the case	20
9. Definitions and methods of analysis of the samples from April 1947	22
10 Literature	23
11. Survey of all analyses of water from the Tim Aa area 1943—48	25

Summary: A pond farm in West Jutland was run without mishap for about 35 years until the discharge of very acid and highly ferriferous water from a lignite pit 11 km up the buffer-deficient watercourse periodically caused a catastrophic mortality among the trout, and the pond farming had to be given up. Also the free water fishery was ruined.

1. The river Tim Aa, situation and surroundings. The river Tim Aa in West Jutland (Fig. 1) drains an area reaching from the surroundings of Grønbjerg-Ørnhøj in the east to the Stadil Fjord and the river Vondaa in the west; the river is formed by the confluence of two brooks, the Spaabæk and the Pølbæk, and later on receives affluents on its northern side from the Thorsted plantation and on its southern side from the marshy areas west of Grønbjerg (partly through the Pølbæk) and from the Hoverdal plantation. The drainage area thus mostly consists of plantations of conifers, stretches of heath on highly washed-out, sandy bottom and marshy areas; clay occurs scantily, for instance on the southern side of the river near the village and church of Thorsted.

In accordance with the character of the surroundings the water of the river Tim Aa is very soft and possesses a low alkalinity only, but these facts did not incommode the fishery until recent times. On the river there was formerly an extensive angling for trout and rainbow trout, and besides this there was a trout pond farm at the Thorsted water-mill —the only one at the river. This pond farm, called the Nr. Esp trout pond farm, was established with 3 ponds in 1906; in 1944 it had gradually grown to a total of 40 ponds covering an area of about 3 hectares and yielding an annual production of about 35,000 kg of trout.

The precipitation area for the part of the river that is situated east of the Nr. Esp pond farm has been authoritatively measured to cover about 4,140 hectares; about 1,470 of them belong to the brook Pølbæk, about 780 to the brook Spaabæk, and of the latter area about 640 hectares drain into the part of Spaabæk that is situated east of the drain of the lignite pit mentioned below.



(Fig. 1. Eastern and central course of the river Tim Aa. 1. Electricity works at Tim. 2. Water mill at Thorsted and the Nr. Esp trout pond farm. 3. The Thorsted-Ulfborg road. 4. The vicarage of Thorsted. 5. Boundary between the parishes of Thorsted and Nr. Omme. 6. The lignite at the brook Spaabæk.)

2. The catastrophes at the pond farm and the investigations in 1943–1944.

The Nr. Esp pond farm was run without mishap from 1906 to 1941, and among pond farmers it was considered one of the best pond farms of this country. In March 1941 it was re-stocked after the lapse of about one year caused by certain difficulties arising from the war situation. About a fortnight after their liberation part of the fish died, but this fact did not arouse suspicion because the owners thought that the fish had been hurt by ice. However, the mortality continued—although to a smaller extent—until September 1943 when a heavy mortality occurred. The same thing took place in October of the

same year, and about December 1st, 1943 the mortality became so disastrous, that practically all the remaining trout of the pond farm were killed. Matters were now more closely examined, partly by the Danish Biological Station and the Experimental Laboratory of the Board of Fisheries, partly (somewhat later) by the present authors as expert members of an agricultural commission appointed for this special purpose.

The pond farmers had observed that the character of the river water had gradually changed: before 1941 it was clear and pure, but from this year onwards the water became more and more turbid from suspended, reddish-brown particles. This was particularly conspicuous by strong flow of water or when the water of the river was stirred by stormy weather. The cases of mortality in 1943 coincided with such a flow of turbid water in the river. An examination of the meteorological data from this tract (Fig. 2) shows that immediately before the catastrophe of about December 1st, 1943 there had been heavy downpours at Nørre Omme within the precipitation area of the river Tim Aa.

Also the lease-holders of the game-fishery of the river had noted this change and in September 1943 sent in a number of water samples for examination; at the same time they called attention to the fact that a lignite pit about 2.5 km west of Ørnhøj discharged water into the brook Spaabæk, an affluent of the river Tim Aa. The water samples showed the following data:

	Locality of sampling				
	Drain of the lignite pit	The Tim Aa at Vinkælder	The Tim Aa at the Thorsted vicarage	The Tim Aa at the Thorsted dairy	The Tim Aa at Søndre Esp
km from the drain of the lignite pit	0	6.4	7.6	8.7	13.4
pH	5.4	5.8	5.8	6.4	6.8
Total hardness ..	6.0	2.5	2.0	2.0	3.0

In other words it was evident that acid water was discharged from the lignite pit and that the river was distinctly acid as far downwards as the vicarage of Thorsted and finally that the reaction of the water as far downwards as Sdr. Esp was still below the neutral point (presumably on account of the carbon dioxide dissolved in the water).

After the catastrophe at the pond farm about December 1st, 1943 a water sample was collected at the Nr. Esp pond farm on December 4th, 1943 by one of the pond farmers and the fishery assistant. On the day of the catastrophe the water had been very turbid, but when the sample was collected nothing abnormal could be seen. The analysed sample showed a pH of 6.7 and an iron content of 2 lmg; the consumption of oxygen with potassium permanganate measured 4.4 lmg. None of these figures indicated that the water was noxious to the fish, but it must be emphasized that the sample was collected not during but after the catastrophe.

After the catastrophe in December 1943 the ponds were re-stocked, but the mortality continued. A water sample collected on March 7th, 1944 by one of the pond farmers in the drain of the lignite pit showed the following data[1]):

Color	Smell	pH	lmg of iron	Comsumption of oxygen with potassium permanganate
Brownish	Normal	5.7	3	75 lmg

Together with the water sample 4 trout were sent in from the Nr. Esp pond farm. They were examined

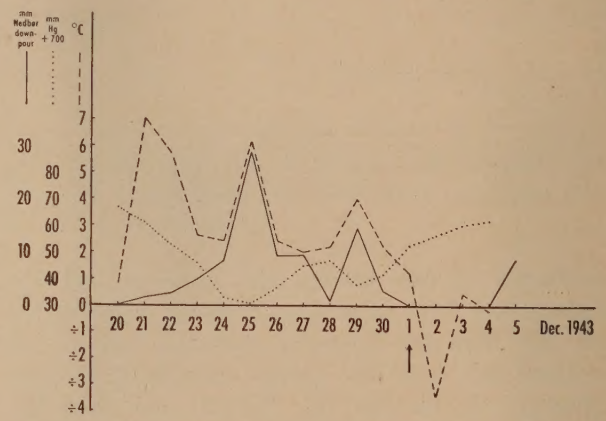


Fig. 2. Conditions of weather around the river Tim Aa before, during and after the catastrophe at the Nr. Esp pond farm on Dec. 1st, 1943. The arrow denotes the day of catastrophe. The downpour measured at Nr. Omme, stated for the previous 24 hours. Height of barometer (altitude above sea-level 54.6 m) and temperature measured at Studsgaard, 34 km from the pond farm at 8 a. m.

at the Biological Station and showed so considerable accumulations of ochre on the gills, that there could be no doubt that the illness of the trout must be caused by this fact ("ochre suffocation").

The iron salts that caused the accumulations of ochre in the gills of the fish were supposed to origin-

[1]) A sample collected at the same time at the drain of the dairy (cp. the survey pp. 24—25) must be supposed to be without interest on account of the development of matters.

ate from the drainage water of the lignite pit, but when analysed on March 7th, 1944 this water had not contained iron enough to support this hypothesis.

On April 3rd, 1944 the Biological Station examined the conditions in the river and at the pond farm. During the previous days a great many fish had died and were still lying in the ponds together with a smaller number of dying fish. Part of the fish were examined; all of them showed accumulations of ochre in the gills. Throughout the area examined—from Thorsted to the pond farm—the river bed was covered by a thick layer of ochre, and the water was milky from small, white particles, which formed slimy coatings in the inlet pipes of the ponds. Above the drain of the Thorsted dairy the oxygen content of the river water was 8.4 ml/l (saturation 94 per cent.), about 10 m below this drain it was 8.3 ml/l (saturation 93 per cent.) and at the inlet of one of the ponds it was 8.4 (saturation 95 per cent.). So the trout of the pond farm could not possibly have been suffocated by lack of oxygen in the river water.

The measurings of pH in the river and the drainage water from the lignite pit gave the following data:

	Locality of samp'ing			
	Drain of the lignite pit	The Spaabæk below the pit, about 300 m	The Tim Aa at the Thorsted dairy	The Tim Aa at the Nørre Esp pond farm
km from the drain of the lignite pit	0	0.3	8.7	11.0
pH	4.2	5.4	5.8—6.0	6.6

and the iron content of a water sample from the drain of the lignite pit was determined to 131 lmg. A water sample from the pond farm sent in about the same time showed a pH of 6.8 and an iron content of 19 lmg. This analysis thus makes it likely that the ochre suffocation of the trout of the Nr. Esp pond farm can be traced to the discharge of ferriferous water from the lignite pit at the brook Spaabæk; but at the same time a comparison of this analysis with the above-mentioned from March 7th, 1943 showed that the nature of the lignite water, more especially its content of iron, was subject to considerable fluctuations, assuming that the samplings were made in the same way at the same locality.

The mortality among the fish of the pond farm was still above the normal during the following time, and in December 1944 another catastrophe brought about the death of 95 per cent. of the remaining stock of trout. Measures against similar catastrophes in the future were discussed in July 1945 by the Inspectorate of Waters in the parish of Thorsted, the verdict of which was carried up to the agricultural commission in the autumn of the same year. In the meantime—on July 17th—18th, 1945—another heavy mortality occurred after downpour (Fig. 3); at the beginning of August the few surviving fish were transferred to another pond farm, and the Nr. Esp pond farm was given up temporarily.

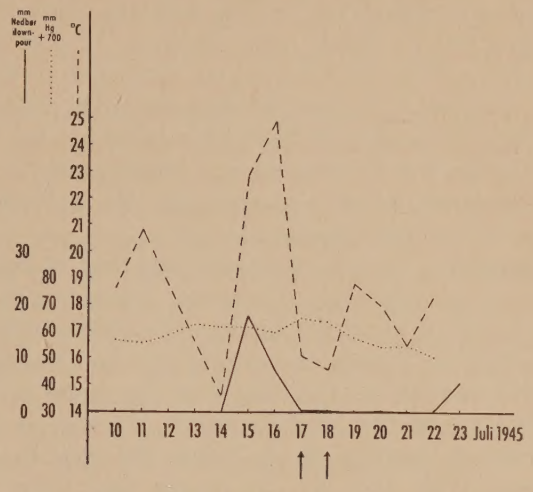


Fig. 3. Conditions of weather around the river Tim Aa before, during and after the catastrophe at the Nr. Esp pond on July 17th—18th, 1945. The arrows indicate the days of catastrophe. The downpour measured at Røddinglund (18 km from the pond farm), stated for the previous 24 hours. Height of barometer (altitude above sea-level 54.6 m) and temperature measured at Studsgaard (34 km away) at 8 a. m.

3. Ochre suffocation and its causes.

Ochre suffocation is by no means a rare disease. Schäperclaus describes it (1 and 2) and mentions that inlets to trout ponds and hatching houses must absolutely be cleared from greater quantities of iron. Also in this country the disease was often observed to result from discharges of acid, ferriferous water into streams at which pond farms are situated; C. V. Otterstrøm (4) and Werner Christensen (5) (cp. H. Pape (6)) have described one of the cases, in which, however, the effect of a high acidity seems to have predominated over the simultaneous effect of a great content of iron.

As a matter of courses most of the Danish instances of ochre suffocation known to us originate

from acid and ferriferous water from moors, but the Biological Station has examined one case in which the noxious water came from a lignite pit —though, as in the case mentioned above, the poisoning by acid seems to have predominated over the ochre suffocation. The German freshwater fishery to a great extent suffers from discharges of acid and ferriferous water from lignite pits, which appears from the fact that Czensny, starting from the influence of these discharges on the fishery has subjected the whole question concerning the interdependence of the hydrogen-ion concentration and the content of iron to a closer investigation (7).

In organic deposits like lignite and peat there are often some sulphurous compounds originating from the plants of which the lignite or peat were formed. The sulphur mostly occurs in the form of pyrites (FeS_2), concretions of which were also found in the lignite pit at the brook Spaabæk. As long as the sulphurous compounds are below the level of the ground water they do not change their character because an addition of oxygen is necessary for that. If, however, the level of the ground water is lowered (cp. 15)—in the moors by draining and in the lignite pit by pumping out water—the air will penetrate through the pores originally filled with water down to the pyrites, which oxidizes according to the equation (5):

$$FeS_2 + 7\,O + H_2O = FeSO_4 + H_2SO_4$$

pyrites + oxygen + water = ferrous sulphate + sulphuric acid.

By continuous airing the ferrous sulphate may oxidize further according to the equation (6):

$$6\,FeSO_4 + 3\,H_2O + 3\,O = 2\,Fe(OH)_3 + 2\,Fe_2(SO_4)_3$$
$$2\,Fe_2(SO_4)_3 + 2\,H_2O = 2\,Fe_2O(SO_4)_2 + 2\,H_2SO_4.$$

During periods of drought the compounds formed in this way will be stored, so that the layers of earth contain a diversity of matters, some of which will be washed out by heavy showers of rain. A solution of ferrous sulphate will give an acid reaction, which will be further accentuated by the presence of sulphuric acid.

It will be understood from this that in certain categories of lignite water and drainage water from moors there may be considerable fluctuations in the contents of iron and acid, these two factors varying in the water not only according to the amount of iron and sulphur contained in the individual layers (in the case of lignite pits both in the pit itself and in the immediate vicinity from which the ground water is drawn by the pumps), but also to a great extent according to the amount, frequency and duration of the downpours. During a drought, when the compounds formed are not washed out but stored, we will presumably get water that is poor in iron and acid, the amounts of which, however, must be supposed to reach formidable heights during heavy downpours after a drought of some length. If the rain continues for some time, the matters formed during the drought will be totally or partially washed out, and new ones are hardly or only slowly formed because the pores of the earth are now filled with water instead of air; the contents of iron and acid in the drainage water may thus decrease during a long-continued period of downpour. Parallel to such fluctuations in the amounts of iron and acid in the drainage water we shall of course find varying effects on the fish population of the stream into which the water is drained. The length of the area in which damage is done is particularly subject to variations: under unfavourable conditions a mortality among the fish may occur in localities situated farther away from the drain, where the fish may well live under less unfavourable conditions.

Figures 2 and 3 are fine illustrations of this accordance between conditions of weather and the occurrence of mortality among the fish of the Nr. Esp pond farm situated 11 km from the lignite pit.

Coincidently with the neutralization of the acidity and the oxidization of the water the iron compounds discharged into the watercourse will be converted into the practically insoluble ochre (ferric oxide), which is harmless to the fish. Quite another thing is that the precipitated ochre in covering the bottom and the surface of the aquatic plants in a purely mechanical way reduces the production of food animals in the stream.

The time employed in the precipitation of the dissolved iron salts in the form of ochre and, accordingly, the length of the stream in which the fish may be hurt among other things depend on the power of the water to neutralize acids, *i. e.* its alkalinity. That the distances are sometimes long appears for instance from Czensny's (7) experience that the precipitation of the iron salts occurs comparatively slowly (cp. the titration of a water sample from the lignite pit described on p. 19). The noxious

effect of the ferriferous water on the fish is due to an accumulation of iron on the gills of the fish as a layer of ochre, which prevents the diffusion of oxygen from the water to the blood-vessels of the gills. Experiments (1) have shown that only 1.9 lmg of iron in water with a pH of 6.7 gave accumulations of iron (though not fatal) on the gills of tench and pike, while a slightly higher content of iron (concentration not stated) had a deadly effect.

4. The free water fishery in the river Tim Aa 1942–45.

Also the fishery in the river was perceptibly influenced by the drainage water from the lignite pit. From the boundary between the parishes of Nr. Omme and Thorsted to the electricity works at Tim the fishery was leased to an association, which in the years 1942—45 took accurate statistics of the catches with information on the localities of the river in which the captures were made. The following Table 1 is based on these statistical data. Section 1 includes the area from the parish boundary to Vinkælder, section 2 the area from Vinkælder to the Thorsted-Ulfborg road, section 3 the area from this road to the Nr. Esp pond farm and section 4 the area from the pond farm to the electricity works at Tim.

As will be seen there is a distinct reduction of the catches, and the reduction has spread gradually from east to west in the direction from the drain of the lignite pit; in section 4 alone, which is situated at the greatest distance from the drain, the population has nearly maintained the 1942 level. The rise of the catches from 1942 to 1943 is caused by a sudden abundance of rainbow trout, which have no doubt escaped or been let out from the ponds. But this increase of the fish population of the river was not for very long able to counterbalance the fall of the total catch. By the end of 1945 nearly all free water fishery within the said areas had ceased.

Table 1. Catches of brook trout and rainbow trout in the Tim Aa 1942—45.

Section no.	1		2		3		4		Total catch	
Year	No.	kg	No.	kg	No.	kg	No.	kg	No.	kg
1942	6	2.9	15	5.7	82	30.5	53	26.4	156	65.5
1943	5	2.0	32	9.2	232	60.6	490	126.7	759	198.5
1944	0	0	2	0.4	16	9.2	112	56.7	130	66.3
1945	0	0	0	0	5	3.3	57	29.2	62	32.5

5. The investigations in 1945–46, the end of the breaking of lignite in June 1946 and the catastrophe in January 1947.

On December 17th, 1945 the holder of the lignite pit had a number of water samples collected at the

Table 2. Reaction, contents of iron and sulphate etc. in the area of the Tim Aa on 17-XII 1945 after rain[1]).

Sample no.	km from the drain of the lignite pit	Time	Locality of sampling	Appearance of water	pH	lmg iron	Sulphate lmg SO_4 ÷ ÷	Consumption of oxygen (lmg) (filtered water)
1		12.00	Discharge from the pumping pipe of the lignite pit	clear, colourless	4.17	260	1461	
2		11.30	Draining ditch abt. 50 m above its fall into the Spaabæk .	greyish-brown, turbid..	3.77	260	1460	3.8
3		11.35	The Spaabæk 25 m above the drain of the pit	greyish, turbid	5.68	1.3	27	7.3
4	0.04	11.45	The Spaabæk 40 m below the drain of the pit.....	like 3	4.68	12.2	90	6.0
5	1.9	12.40	The Spaabæk 125 m above its confluence with the Pølbæk	greyish, turbid	4.47	5.1	80	4.4
6		12,50	The Pølbæk 75 m above its confluence with the Spaabæk	turbid	4.74	6.5	44	5.8
7	2.025	13.00	The Tim Aa abt. 25 m below the confluence of the Spaabæk and the Pølbæk	somewhat purer than 6	4.89	3.8	59	4.1
8	6.4	13.50	The Tim Aa at Vinkælder	brownish.....	4.75	13.3		
9	8.7	13.45	The Tim Aa at Thorsted dairy	more brownish than 8 .	5.01	19.4		
10	11.0	14.10	The Tim Aa at the Nr. Esp pond farm.....	more brownish than 9.	5.97	20.4		
11		13.40	Drain from Thorsted dairy, water nearly stagnant	slightly turbid, colourless	6.55	1.4		

[1]) Collected and analysed by Quist's Laboratory, Aarhus. We are indebted to Mr. T. A. Thomsen, C. E., and the holder of the lignite pit for the permission to publish these analyses.

pit, in its drain and in the river and some of its affluents. The results of the analyses of these samples are found in Table 2.

In the brook Spaabæk above the lignite pit (St. 3) was found a distinctly acid reaction combined with a not abnormal iron content and a small content of sulphate.

The discharge of the very acid water from the lignite pit (St. 1), which, in spite of its clearness, showed an extreme richness in iron and sulphate, made the water below the drain (St. 4) very acid (change of pH from 5.7 to 4.7), and at the same time the iron content rose from 1.3 to 12.2 lmg, the sulphate content from 27 to 90 lmg.

The high degree of acidity continued throughout the rest of the brook Spaabæk, while the iron content fell to 5.1 lmg at St. 5. There was no fall worth mentioning in the content of sulphate.

The water of the brook Pølbæk (St. 6) above the confluence reacted more acidly than the original Spaabæk water, and the iron content was higher than at the lower end of the Spaabæk (St. 5). The sulphate content was about half of that of the Spaabæk (St. 5). Below the confluence of the two brooks the reaction was higher than in any of the two affluents while the iron content was lower. The content of sulphate was lower than at St. 5 and a little higher than at St. 6.

After a slight fall at Vinkælder (St. 8) the pH value rose steadily down the river. At the same time, however, there was a rise of the iron content.

These samples were collected after very heavy showers, *i. e.* at one of the points of time which according to out experience would be dangerous to the stock of fish. During the sampling, however, there has most likely been a suspension of ochre in the water, which is seen from the appearance of the water, so that the determinations of iron cover both suspended (harmless) iron and the dissolved (dangerous) iron compounds.

The lease-holder of the lignite pit maintained—as would be expected—that the pumping water from the pit alone could hardly cause the mortality among the trout in the river and at the pond farm. This contention *i. a.* was based on the low pH values found in these water samples from the Pølbæk and on the iron content of the same locality combined with the fact that the flow of water on the said day was considerably greater in the Pølbæk than in the Spaabæk. At that time the contention could not be waved, but it is highly invalidated by the fact that the suspended, precipitated iron (ochre) is included in the analyses. This ratio is hardly—neither absolutely nor relatively—of the same size-order because the "error" must be supposed to be rising in the zone of the stream that shows the most conspicuous deposits of ochre; so we must not attach importance to the rise of the iron content from the upper end of the river Tim Aa down towards the Nr. Esp pond farm (cp. Table 2), neither must we for the same reason lay weight on the content of iron found in the brook Pølbæk. The rising intensity of the brown colour of the water at stations 8—10 is highly in favour of the supposition that within this very area there has been an increasing suspension of ochre.

About February 22nd—24th, 1946 one of the pond farmers collected 2 samples of water from the lignite pit, one clear and one turbid sample, taken at times, respectively, when no pumping took place and when water was being pumped from the pit. One set of samples was analysed by the Experimental Laboratory of the Board of Fisheries and a corresponding set—apparently collected one or two days later—by Quist's Laboratory at Aarhus. The results were as follows:

		pH	lmg iron	Sulphate lmg SO_4÷÷
1. Series	Clear sample ..	3.72	165	..
	turbid sample .	3.51	255	..

		pH	lmg iron	Sulphate lmg SO_4÷÷
2. Series	Clear sample ..	4.12	250	1274
	turbid sample .	3.95	260	1376

In other words it had now been ascertained

i. that highly acid water with a very great content of sulphate and iron is occasionally discharged from the lignite pit at the brook Spaabæk;

ii. that there was a more or less acid reaction of the water of the river Tim Aa from the lignite pit to the Nr. Esp pond farm and that the water at the pond farm was often highly coloured by ochre, which was not the case before the working of the lignite pit was started;

iii. that the fishery in the free water of the river had decreased very much within the areas situated in the vicinity of the lignite pit;

iv. that the Nr. Esp pond farm had to be given up on account of an occasionally catastrophic mortality among the trout; and
v. that the mortality among the trout in the cases examined was due to ochre suffocation.

It was an obvious conclusion that the troubles were connected with the water pumped out from the lignite pit—the more so because no troubles of a chemical nature had been observed in the river or at the pond farm before the working of the lignite pit was started.

As pointed out above the trout of the Nr. Esp pond farm died from ochre suffocation. On April 11th, 1946 one of the authors (K. L.) put out 3 one-year-old fingerlings of rainbow trout in a corf in the brook Spaabæk about 125 m above its confluence with the brook Pølbæk. At this point of time the water was somewhat turbid but clearing, so that about 3 hours later it was as clear as crystal. At this time two of the fish were lying on their sides showing distinct signs of respirational difficulties and of beginning coagulation of slime. One fish was taken out, the other two remaining in the corf. About 6 hours after they had been put out they were found to be dead, and the slime of their skin was grey and coagulated. Moreover there were very distinct accumulations of ochre on their gills. For the sake of control 3 rainbow trout in a corf had been put out in the Spaabæk above the drain of the lignite pit, and about 10 rainbow trout had been put out in the Pølbæk above the place where it joins the Spaabæk. Both sets of control fish were intact when the experiment was stopped after 7 and 6 hours, respectively. Some water samples collected during the experiments showed the following data:

Locality of sampling	pH	iron mg/l
The Spaabæk above the lignite pit ..	7.18	3.5
Drainage water from the pumps of the lignite pit	4.12	110
The Spaabæk abt. 125 m above its confluence with the Pølbæk	4.35	52.5
The Pølbæk 75 m above its confluence with the Spaabæk.....	7.15	3.5

The enormous fall of pH and the simultaneous rapid rise of the iron content of the Spaabæk below the drain of the lignite pit are conspicuous, and the result of the experiment with the trout is by no means astonishing.

In June 1946 the working of the lignite pit ceased, and conditions in the river would then be expected to improve. For the purpose of verifying this an examination was made on September 6th—7th, 1946. The pumping out of water from the pit had ceased long ago, but from the large heaps of overburden that covered an area of about 5.8 hectares (Fig. 4) there was a constant oozing out of water that gathered into a stream carrying roughly 3—5 l/sec. and draining into the Spaabæk through the ditch of the former drain. As will appear from the results of analyses of the water samples from September 6th, 1946 given in Table 3 the water of the ditch was very acid and—in spite of its clearness—had a considerable content of iron.

For the first time since the beginning of the investigations we thus find neutral water at the Nr. Esp pond farm, even at a time when the brook Spaabæk above the drain of the lignite pit and the Pølbæk carried acid water and while there was still an outflow of very acid water from the area of the lignite pit. The result of this examination seemed to give hopes of a new start of the pond farm without any great risk, a hope, however, which proved to be false.

The pond farmers were adviced to re-stock the ponds by degrees, but unfortunately the advice was not taken. By the end of November 1946 231,000 six-months-old fingerlings and 3,550 kg of fish below the size-limit and mother-fish were put out at the Nr. Esp pond farm where they did well for about 6 weeks until January 4th, 1947. But on the night of this day another catastrophe occurred: 163,400 pieces of fingerlings and 1,305 kgs. of bigger fish died. The cause of death was not examined in detail in this case, but there is a report from the Fishery Patrol to the effect that the gills of the fish were covered by "a brown, slimy substance."

Under the assumption that the catastrophe in January 1947 is due to ochre suffocation, which must no doubt be supposed to be the case, it is quite exceptional as regards conditions of weather because it did not occur after heavy rain, but after and during a period of frost (Fig. 5). The explanation of this fact is not quite clear. A possible explanation is that the frost has bound superficial affluents, rich in buffer (for instance from the occurrence of clay at Thorsted) while it was unable to bind the water from the area of the lignite pit (which freezes

Table 3. Reaction and iron content of the Tim Aa area on 6-IX-1946 after rain.

Sample no.	Locality of sampling	Appearance of the water	pH	iron lmg
1	Draining ditch above its fall into the Spaabæk	clear	3.00	66
2	The Spaabæk abt. 25 m above the outfall of the draining ditch	somewhat turbid	5.90	12
3	The Spaabæk abt. 125 m above its fall into the Tim Aa	turbid, partially from suspended ochre	3.55	14
4	The Pølbæk 75 m above its confluence with the Spaabæk	brown, from suspended ochre	4.80	24
5	Affluent from the north to the Tim Aa through the easternmost draining pipe at Abisgade	clear; roughly 5—6 l/sec.	6.25	8
6	The Tim Aa at the Nr. Esp pond farm	clear with a few small particles, presumably ochre; vegetation green and without ochre	7.0	11

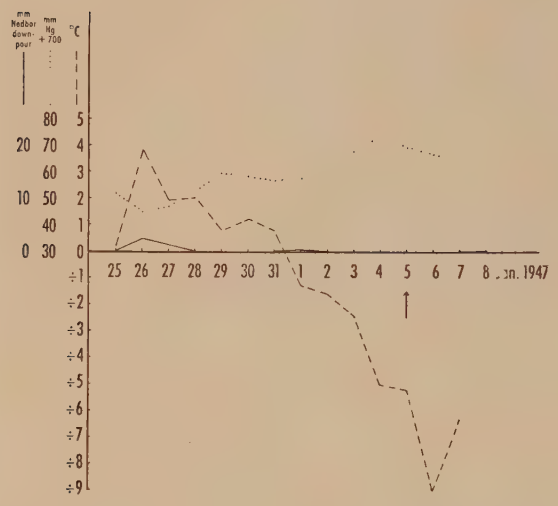


Fig. 5. Conditions of weather around the river Tim Aa before, during and after the catastrophe at the Nr. Esp pond farm on Jan. 5th, 1947. The arrow denotes the day of catastrophe. The downpour measured at Nr. Omme on Dec. 25th—31st, 1946, at Røddinglund (18 km from the pond farm) on Jan. 1st—8th, 1947. Height of barometer (altitude above sea-level 54.6 m) and temperature measured at Studsgaard (34 km from the pond farm) at 8 a. m.

at a sligthly lower temperature than pure water on account of its higher salinity), which thus came to make itself felt for a longer distance than "normally"; local climatic conditions are possibly involved. But of course all these explanations are only conjectures because no examination was carried out simultaneously with the catastrophe, which was not learned of by the authors until about two months after it had happened.

6. The analyses from April 1947.

On account of the severe winter-weather it was impossible to carry out examinations at the place immediately after information of the catastrophe in January 1947 had been received. On April 23rd, however, the Biological Station received a series of water samples from the drain of the lignite pit and the Tim Aa, taken by one of the pond farmers. The analyses yielded the figures given in Table 4.

It appears from this that after the brook Spaabæk has been joined by the highly acid and very ferriferous water coming from the heaps of overburden at the lignite pit its water has changed from a normal, buffer-deficient water with quite a normal ion concentration (specific conductance) into a highly acid, ferriferous water, without alkalinity and with an acidity of a very high size-order as compared to normal surface water. Also the water of the brook

Table 4. Analyses of water samples from the Tim Aa area, 21-IV-47.

Locality of sampling	Reaction pH	Specific conductance $\varkappa_{20} \cdot 10^6$	Iron lmg Fe	Alkalinity mval/l	Acidity mval/l
A. The Spaabæk above the lignite pit	6.1	173	1.5	0.22	—
B. Drainage water from overburden of the lignite pit	3.1	2600—3100	200	0	21.2
D. The Spaabæk below the drain of the lignite pit	3.4	520	12	0	0.85
E. The Pølbæk, lower end	4.4	226	4	0	0.15
K. The Tim Aa at the Nr. Esp pond farm ...	6.6	191	1.5	0.52	0

Fig. 4. Air photography of the upper part of the river Tim Aa and the brooks of Spaabæk and Pølbæk. 1. The Tim Aa. 2. The Spaabæk. 3. The Pølbæk. 4. The lignite pit with the overburden placed in walls. 5. The village of Grønbjerg (1:20 000).

(Geodætisk Institut, copyright.)

Pølbæk has a low pH value, is without alkalinity though its acidity does not exceed the values found elsewhere in normal surface waters. At the Nr. Esp pond farm the reaction of the water was slightly acid; an examination showed that this was due to carbon dioxide because the reaction rose to pH 7.6 after the carbon dioxide had been expelled. Here the specific conductance showed no abnormal ion concentration (only slightly higher than in the upper course of the Spaabæk), neither was the iron content remarkable. Finally a distinct content of bicarbonates (alkalinity) could be established. On the day of sampling, therefore, this water must be considered sound water in as far as these analyses allow such a conclusion.

Because these samples had been collected by one of the parties of the case pending one of the authors (K. L.) on April 30th, 1947 collected 10 samples, which were sent to the other author for analysis.

The following is a detailed discussion of this series of analyses (Table 5), which has brought as clear a proof as might be expected of the share of the lignite pit in the catastrophes at the Nr. Esp pond farm and in the demonstrated reduction of the free water fishery in the river:

In the Spaabæk above the drain of the lignite pit (A) the water was found to be slightly acid, buffer-deficient, with a low (but normal) ion concentration, without acidity and with a distinct, though not abnormal content of iron and a very small content of sulphate. When the sample was collected the water was slightly opalescent, and during the transport (about 24 hours) there appeared a slight sediment (precipitated iron). No remarkable scent, neither at ordinary room temperature nor at a cautious heating to about 60° C. (which is also true of all other samples from this day). The analyses gave no justification for characterizing this water as abnormal. On the contrary it must be considered legitimate to consider this water fairly expressive of the state of the water throughout the brook Spaabæk in former times.

The lignite pit (B). When sampled the water was clear and without suspended particles, and on the reception it was still clear and without sediment. When heated to 60° C. it grew yellow and highly opalescent. The water was here highly acid, with a very high ion concentration, without alkalinity, with an abnormally high acidity and abnormally great contents of iron and sulphate. It thus differs decisively from the normal ground water of West Jutland (8, for inst. pp. 44—46, 117—118) and also from other normal ground waters (9, 10). During the case it has been pleaded that this was normal ground water, but no proof has been produced of the correctness or even probability of this contention. At any rate none of the several hundred analyses of Danish ground waters made by Ødum & Werner Christensen (8) shows similar results. It is true that there are many instances of acid waters and some instances of water without alkalinity; it is also possible to find waters with rather great contents of iron and waters with comparatively great contents of sulphates. But it is impossible to find one instance of ground water with a very acid reaction combined with great contents of both iron and sulphate. On the other hand we can hardly exclude the possibility that after the extensive diggings of recent years for lignite and peat you will meet ground water, infected by water that has passed through layers of earth containing great quantities of iron and sulphate that have been exposed to the air, but this is no

Table 5. The Tim Aa area 30-IV-1947.

Locality of sampling	Reaction pH	Specific conductance $\varkappa_{20} \cdot 10^6$	Iron lmg Fe	Sulphate lmg SO_4^{--}	Alkalinity mval/l	Acidity mval/l	Bicarbonates lmg HCO_3^-
A. The Spaabæk above the drain of the lignite pit	5.9	173	3.5	4.6	0.26	0	14
B. Drain from the overburden of the lignite pit	3.4	2655	175	1509	0	20.9	0
C. Lower end of draining ditch from the lignite pit	3.4	1785	90	756	0	10.8	0
D. The Spaabæk below the drain of the lignite pit	4.6	381	90	85	0	0.71	0
E. The Pølbæk, lower end	4 8	202	8.5	23	ca. 0.01	0.13	ca. 0.6
F. Ochre ditch draining into the Pølbæk	6.1	121	3.1	14	0.13	0.02	8
G. The Tim Aa, abt. 100 m below the confluence	4.6	241	10	39	0	0.25	0
H. The Tim Aa at Vinkælder	5.3	191	7.5	17	0.04	0.04	2.5
I. The Tim Aa at the Thorsted dairy	6.4	186	6.2	13	0.15	0	8
K. The Tim Aa at the Nr. Esp pond farm	6.9	181	4.7	7.1	0.53	0	31

justification for calling such ground water "normal".

The draining ditch from the lignite pit, immediately before it falls into the Spaabæk (C). In the ditch were found thin sediments of ochre. When sampled the water was clear, but on the reception it was opalescent, without sediments. When heated to 60° C. it grew yellow and much more opalescent. The water was still very acid and showed a very high ion concentration, no alkalinity and an abnormally great content of iron and sulphate. During the passage of the water through the ditch there has in other words been a considerable reduction of the different factors examined—perhaps not the less owing to the easy chance of absorbing oxygen from the air—but not to a decisive degree, and the water must still be considered abnormal or say pathological. The use of these terms, however, must be viewed from our knowledge of normal ground and surface waters, but they are no doubt far from being abnormal for lignite waters in general.

If the normal water of the brook Spaabæk should be able to neutralize this water from the lignite pit, the flow of water in the Spaabæk must be about 40 times greater than that of the lignite drain, calculated from the analyses given here.

The brook Spaabæk below the lignite pit and immediately above its confluence with the brook Pølbæk (D). Comparatively thick sediments of ochre were found in the brook; the water was opalescent, milky. On the reception was found a flocculent, brownish sediment, and the water was now quite clear. Reaction highly acid, comparatively high specific conductance, no alkalinity, very considerable acidity, abnormally great content of iron and remarkably great content of sulphate.

A comparison between the analyses from the upper and lower parts of the brook Spaabæk will show that the brook water after the reception of the water from the lignite pit has undergone a violent and, in point of principle, very important change. The reaction has decreased to pH 4.6; the alkalinity has disappeared: the previously very small amounts of bicarbonate have been decomposed to carbon dioxide by the acid lignite water, which has thus deprived the water of its natural power of counteracting the fluctuations of pH; instead of this we now find an acidity which, it is true, in comparison to that of the drainage water is much lower, but in comparison to normal water (cp. for instance the following analysis from the brook Pølbæk on the same day) is very high. The maximum of the very few instances of acidity found in natural waters of this country (9) is 0.5 mval/l, which goes well with results not yet published by the present authors. Here the acidity is about twice the height of what must at present be considered the maximum of normal waters. In this connexion it should be mentioned that Boisen Bennike's material is based on titration with phenolphtalein as indicator (to pH 8.3) while the results given here were calculated by electrometrical titration to pH 7.0 (see further p. 22), so that the difference is in fact a little greater than stated here.

It must be emphasized that the amount of iron in this locality is precisely the same as in the drainage water from the heaps of overburden at the lignite pit. As mentioned above it has been calculated—from the alkalinity and the acidity—that the brook Spaabæk must carry 40 times more water than the drain in order to neutralize the water of the latter.

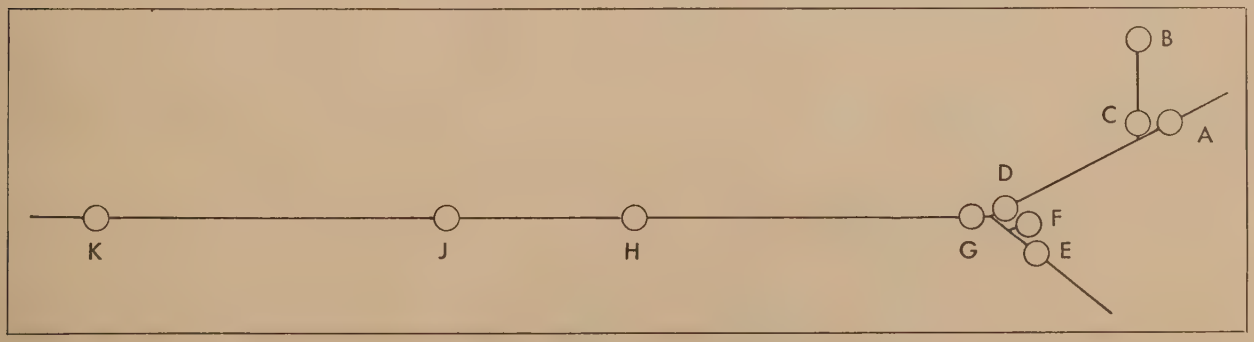


Fig. 6. The Tim Aa area. Schematic presentation of the localities of sampling on 30-IV-47. (A. The Spaabæk above the lignite pit; B. The lignite pit; C. The drain of the lignite pit; D. The Spaabæk below the lignite pit; E. The Pølbæk; F. Ochre ditch draining into the Pølbæk; G. The Tim Aa about 100 m below the confluence of the brooks Pølbæk and Spaabæk; H. The Tim Aa at Vinkælder; I. The Tim Aa at the Thorsted dairy; K. The Nr. Esp pond farm.)

We are able to say that on April 30th, 1947 there was a dilution of only half this size (1:21.5).

The lower course of the brook Pølbæk (E) (but above the following sample). Inconsiderable sediments of ochre on the bottom, the water opalescent, slightly milky. On the reception there was a flocculent sediment, and the water was but slightly opalescent. This sample was taken because it had previously been maintained that this ditch carried acid, ferriferous water and had therefore caused the damage to the fishery. It is true that the water was acid (pH 4.8) and highly ferriferous (8.5 lmg), but the latter value is only about 10 per cent. of the amount of iron found in the lower course of the brook Spaabæk. The alkalinity was found to be very low (0.01 mval/l, on the very limit of what can be established by the rather exact method applied); the acidity was 0.13 mval/l, which is not above the values that may be found in natural, acidotrophic waters.

Ochre ditch draining into the Pølbæk (F) below the previous locality of sampling. When the sample was collected the flow of water was estimated at 20 l/sec. Thick sediments of ochre on the bottom, the water clear. When received the water was still clear, but showed a thin sediment. This ditch was also suspected of carrying acid, ferriferous water, that is to say of being noxious to the fish. The analyses from April 30th, 1947, however, do not corroborate this supposition. The reaction of the water was but slightly acid (pH 6.1), and the content of iron was not higher than is known from natural surface water of other localities. The content of sulphate was small, the specific conductance low (the lowest of all samples); there was a slight but distinct alkalinity and a slight acidity, caused by weak acids (humic acids ?).

It would also be interesting to have had a water sample from the brook Pølbæk below the outfall of this ditch, but it is legitimate to conclude from these two analyses that the water of the brook Pølbæk immediately at the confluence with the brook Spaabæk presumably showed a reaction that was a little less acid (*i. e.* had a higher pH) than stated in the analysis from the Pølbæk; the specific conductance has been a little lower, the alkalinity a little higher, the acidity a little lower and the contents of iron and sulphate have been slightly lower than stated here from the Pølbæk. In other words: on the day in question the water falling out from the Pølbæk has been more favourable to the fish than appears from the analyses.

The river Tim Aa, about 100 m below the confluence of the brooks Spaabæk and Pølbæk (G). The sediments of ochre in the river inconsiderable. The water opalescent, slightly milky. On the reception the water was clear, but had a flocculent sediment. The reaction very acid (pH 4.6); the specific conductance lower than in the lower course of the Spaabæk, but higher than in the Pølbæk. No alkalinity, but an acidity of 0.25 mval/l. An iron content of 10 lmg, which is more than in the Pølbæk and somewhat less than in the lower course of the Spaabæk. Also the content of sulphate (39 lmg) is distinctly greater than in the Pølbæk and smaller than in the lower course of the Spaabæk. It appears concurrently from these results that the water of the upper course of the river Tim Aa is distinctly marked by the pathological water from the brook Spaabæk.

If we calculate the dilution from the acidity, we find that for each litre of water from the Spaabæk there is an outflow of 3.8 litres from the Pølbæk. The dilution calculated from the content of sulphate is 1:3. In other words: on account of its quite abnormal chemical properties the less voluminous flow of the Spaabæk comes to predominate over the more voluminous flow of the Pølbæk.

The river Tim Aa at Vinkælder (H). Some sediments of ochre on the bottom of the river. The water was slightly opalescent. When received the sample was clear, but showed a flocculent sediment. The water of this locality is still distinctly acid, but the reaction is somewhat higher than at the previous station. In comparison to the previous sample the specific conductance has fallen (*i. e.* there is a lower ion concentration); a low, though measureable alkalinity has appeared; the acidity has fallen below the maximum known from natural waters; the content of iron is somewhat reduced, but still very great for surface water; the sulphate content has fallen by more than 50 per cent.

The Tim Aa at the Thorsted dairy (I). Some sediments of ochre on the bottom of the river. The water was opalescent, but clear on the arrival of the sample though it contained a flocculent sediment. As compared to the previous station a not inconsiderable improvement has taken place though the water, from the fisherman's point of view, cannot yet be considered satisfactory in all details. The

Table 6. The Tim Aa area. Principal data from the material of analysis.

Locality	Date	Reaction pH	Iron lmg Fe	Sulphate lmg SO_4 ÷÷	Alkalinity mval/l	Acidity mval/l	Specific conductance $\varkappa_{20} \cdot 10^6$
A. The Spaabæk above the lignite pit...	$^{17}/_{12}$ 45	5.7	1.3	27			
	$^{11}/_{4}$ 46	7.2	3.5				
	$^{6}/_{9}$ 46	5.9	12				
	$^{21}/_{4}$ 47	6.1	1.5	+	0.22		173
	$^{30}/_{4}$ 47	5.9	3.5	4.6	0.26	0	173
B. The lignite pit	$/_{9}$ 43	5.4					
	$^{7}/_{3}$ 44	5.7	3				
	$^{3}/_{4}$ 44	4.2	131				
	$^{17}/_{12}$ 45	4.2	260	1461			
	—	3.8	260	1460			
	$^{22}/_{2}$ 46	3.7	165				
	—	3.5	255				
	$^{24}/_{2}$	4.1	250	1274			
	—	4.0	260	1376			
	$^{11}/_{4}$ 46	4.1	110				
	$^{6}/_{9}$ 46	3.0	66				
	$^{21}/_{4}$ 47	3.1	200	+++	0	21.2	2600—3100
	$^{30}/_{4}$ 47	3.4	175	1509	0	20.9	2655
	—	3.4	90	756	0	10.8	1785
D. The Spaabæk below the drain of the lignite pit.....	$^{3}/_{4}$ 44	5.4					
	$^{17}/_{12}$ 45	4.7	12.2	90			
	—	4.5	5.1	80			
	$^{11}/_{4}$ 46	4.4	52.5				
	$^{6}/_{9}$ 46	3.6	14				
	$^{21}/_{4}$ 47	3.4	12	+	0	0.85	520
	$^{30}/_{4}$ 47	4.6	90	85	0	0.71	381
E. The Pølbæk.....	$^{17}/_{12}$ 45	4.7	6.5	44			
	$^{11}/_{4}$ 46	7.2	3.5				
	$^{6}/_{9}$ 46	4.8	24				
	$^{21}/_{4}$ 47	4.4	4	+	0	0.15	226
	$^{30}/_{4}$ 47	4.8	8.5	23	ca. 0.01	0.13	202
G—H. The Tim Aa between the confluence of the brooks Spaabæk and Pølbæk and Vinkælder	$/_{9}$ 43	5.8					
	$^{17}/_{12}$ 45	4.9	3.8	59			
	—	4.8	13.3				
	$^{30}/_{4}$ 47	4.6	10	39	0	0.25	241
	—	5.3	7.5	17	0.04	0.04	191
K. The Tim Aa at the Nr. Esp pond farm	$^{4}/_{12}$ 43	6.7					
	$^{3}/_{4}$ 44	6.6–6.8	19				
	$^{17}/_{12}$ 45	6.0	20.4				
	$^{6}/_{9}$ 46	7.0	11				
	$^{21}/_{4}$ 47	6.6	1.5	+	0.52	0	191
	$^{30}/_{4}$ 47	6.9	4.7	7.1	0.53	0	181
	$^{2}/_{1}$ 48	6.8	12		0.3		
	$/_{1}$ 48	6.2	12.5		ca. 0.1		

reaction is steadily rising (pH 6.4); there is a slight fall of the specific conductance; the alkalinity has risen (though it is still lower than in the upper course of the Spaabæk); the acidity has disappeared (which means that the slightly acid reaction was caused by carbon dioxide); the contents of iron and sulphate have descreased, but it must be emphasized that the amount of iron is still alarmingly great.

The river Tim Aa at the Nr. Esp pond farm (K). Some sediments of ochre on the bottom, the water slightly opalescent, clear on the reception, but with a slight sediment.

The advancing improvement of the character of the water is distinct: the reaction is very close to the neutral point, there is another slight fall of the specific conductance, a distinct content of bicarbonates (alkalinity), no acidity and somewhat less iron and sulphate. To the fish, however, the iron content is still above the critical limit mentioned by Schäperclaus (1), provided that we have to do with dissolved iron salts.

7. Principal data from the material analysed.

The previous pages contain a detailed account of the most important items of information on the nature and extent of the catastrophes, and through an examination of the meteorological conditions and analyses of a number of water samples we have tried to show that the cause of the catastrophes is the discharge of the very acid water with its great contents of iron and sulphate originating from the lignite pit and the heaps of overburden.

These samples were taken irregularly as regards both time and place, and they are incongruous as regards the extent of the factors examined.

In the following we will try to find out what may be deduced from this heterogeneous material as regards the chemical composition of the water in the principal localities of the Tim Aa area. A synopsis of the material is found in Table 6, which contains the analyses from the brook Spaabæk above the lignite pit; the lignite pit (including the drain from the pit and, after the working had ceased, from its overburden); the brook Spaabæk below the pit; the brook Pølbæk; the river Tim Aa between the confluence of the two brooks and Vinkælder; and finally the Tim Aa at the Nr. Esp pond farm. The analyses are given chronologically for each of these areas of sampling.

The brook Spaabæk above the lignite pit (A). It will be seen that the reaction was highly fluctuating (pH 5.7—7.2); in most cases it was below the neutral point, which must be due to the carbon dioxide alone because no other acids than carbonic acid could be found on April 30th, 1947. At any rate it is a safe assumption that other acids were never present in more than negligible quantities. The water from this locality seems to correspond to the waters of Danish lakes described by Iversen (11) as fluctuating highly-slightly acid waters, the very characterization of which is that they are buffer-deficient (*i. e.* have a low alkalinity). As mentioned above the analyses from this locality on the whole must be considered expressive of the nature of the water throughout the brook Spaabæk as it would have been if the drainage from the lignite pit had not radically changed its character. As a rule the content of iron was not very remarkable. The rather considerable amount of iron (12 lmg) found on September 6th, 1946 is presumably due to suspension of ochre because the sample was taken after rain and while the water was turbid.

The content of sulphate, the alkalinity, acidity and specific conductance do not give rise to other comments than those found on p. 14.

The lignite pit (B). When first examined (Sep. 1943 and March 1944) the reaction of the water of this locality was pH 5.4 and 5.7, but after that time it always ranged between pH 3.0 and 4.0. Of course this does not preclude the possibility of similar low values of the reaction on previous occasions, but there is no proof of this—and by the nature of the case no such proof can be found.

The first examination of the content of iron (March 1944) gave only 3 lmg, and—as emphasized—this amount does not allow us to consider the outlet of water from the lignite pit the cause of the calamities. But it must be emphasized that this analysis was made at a time when the mortality at the pond farm was not catastrophic. It is remarkable that already one month later the iron content proved to be 131 lmg, and after that time it has ranged between 66 and 260 lmg. For natural waters these figures would be quite abnormal.

Assuming that the sampling was reliable it remains to record that at the beginning of the investigation the content of iron was subject to great fluctuations and at times was of quite a normal size-order. But not one analysis from the last 3 or 4 years supports the hypothesis that this is still the case. A possible explanation of this peculiar fact is that the winning of lignite over vaster areas and at greater depths gave larger "mountains" of overburden, and that the pumping off of water from the lignite pit has been proved to increase since December 1943.

Since the first analysis (December 1945) the content of sulphate has been great.

No alkalinity could be established.

The abnormally high figures for acidity established in April 1947 are very characteristic. As will be

shown later on the acidity was determined electrometrically by adding rising quantities of sodium hydroxide (n/10 NaOH) to the measured water sample after the carbon dioxide of the air had previously been driven out and determining the resulting p H values.

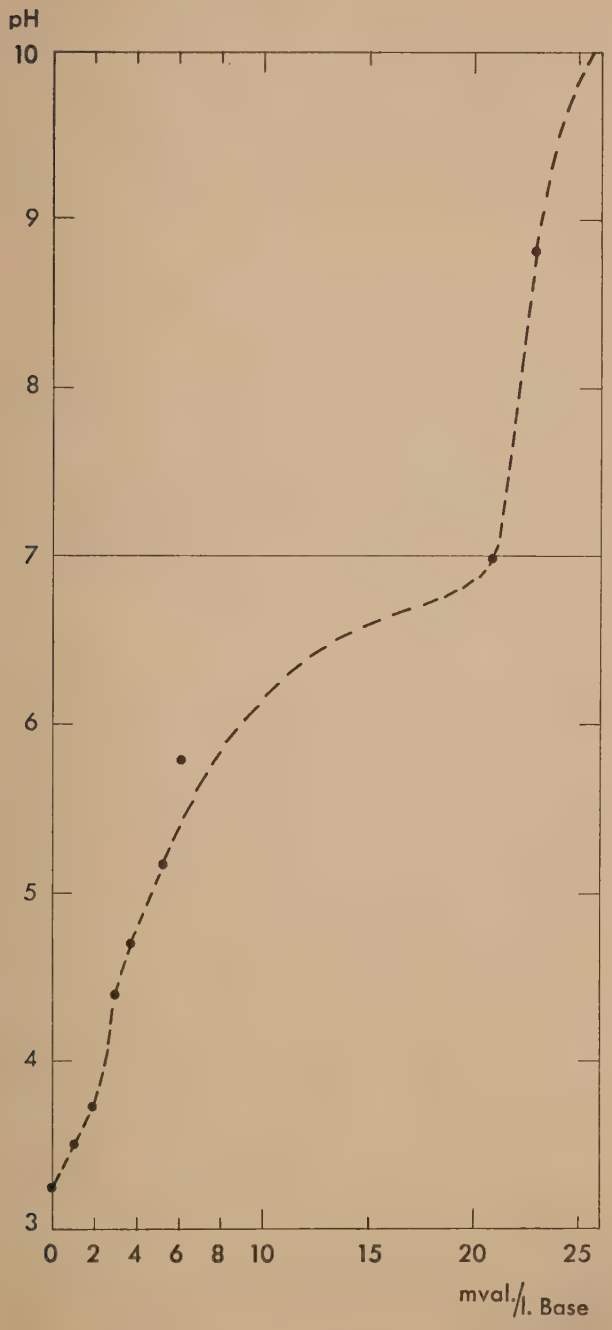


Fig. 7. Titration for acidity in water from the lignite pit on 30-IV-47. See text.

The following is an instance of the progress of one of these titrations (cp. Fig. 7, the water sample collected at the lignite pit on April 30th, 1947).

After the adding of about 5 ml of base the reaction was about pH 5, after which it grew more and more difficult to fix the desired points of the curve up to pH 7 (read as pH 6.97). After the addition of about 6 ml of base (6.17 mval NaOH) pH was read to 5.97, but the balance appeared very slowly, and the reading of this value cannot therefore be claimed to be exact. After this the water grew flocculent and yellowish-brown, and, with rising addings of base, it turned successively darkgreen and dark reddish-brown. Within this area the run of the curve has therefore been plotted by estimate. If the acid reaction had been caused by sulphuric acid alone, the water would have been neutralized by much smaller amounts of base, and so the run of the curve shows that the water contained some matters that were very difficult to neutralize. The principal ones are presumably the sulphurets of iron, but in the present investigation it is in fact of inferior importance to determine the nature of these matters. The really important fact in this connexion is that the water from the lignite pit was highly acid and could be neutralized only by the adding of very great quantities of base.

After the neutral point had been reached the rest of the curve could be plotted without difficulty.

From Danish waters there are few records on the specific conductance (12, 14), but if we compare them with those known from abroad and from the unpublished experiences of the present authors, we are able to state that the specific conductance is extraordinarily high and quite abnormal for freshwaters. It is only in brackish waters with a content of 500—800 lmg of chloride that we find a specific conductance of this size-order.

The brook Spaabæk below the drain of the lignite pit (D). Apart from the first determination of pH, which here, too, seems to hold an exceptional position possibly because the draining of water from about this point of time took place through the ditch instead of by means of the previous irrigation, it will be seen that the reaction was always very acid, pH fluctuating between 3.4 and 4.7. Apart from this first pH value the reaction was always much lower than in the Spaabæk above the drain. The difference amounted to 1.0—2.8 pH units.

The content of iron seems to fluctuate very much,

the values always being comparatively high, sometimes even very high, amounting to about 25 times the quantities found at the same time in the upper course of the brook.

The content of sulphate was determined in 3 analyses, according to which it was of a constant size-order and much lower than in the water drained from the lignite pit.

An alkalinity was not established, but theoretically it was present when the water was analysed on April 3rd, 1944. Its size-order could not be calculated, but without running the risk of drawing rash conclusions we may consider it legitimate to suppose that on this occasion it was very low.

An abnormally high acidity was found in the two samples from April 1947 (see pp. 15—16).

Quite naturally the specific conductance rose somewhat below the drain of the lignite pit, and its size-order corresponds to the values to be expected in alkaline waters, rich in buffer.

The brook Pølbæk (E). In April 1946 a neutral reaction was observed, but otherwise the pH value was below 5 (4.4—4.8). In September 1946 the content of iron was found to be 24 lmg, but this abnormally large amount is presumably a result of the presence of precipitated ochre—which in this connexion is without importance—beside dissolved iron compounds. The alkalinity was practically = 0, the acidity of no abnormal size-order, and there was nothing abnormal about the specific conductance.

The river Tim Aa between the confluence of the brooks Spaabæk and Pølbæk and Vinkælder (G—H). The reaction was distinctly acid (pH 4.6—5.8) and the content of iron mostly of a remarkably high order though it did not approximately reach the high values established in the lower course of the Spaabæk, which shows the power of the river to precipitate part of the iron. No or only a very low alkalinity was established. An acidity of up to 0.25 mval/l. The specific conductance not remarkable.

The river Tim Aa at the Nr. Esp pond farm (K). The reaction was always lower than or (exceptionally) = pH 7.0 (the neutral point). The content of iron showed great fluctuations and on one occasion was below the dangerous limit mentioned above. On most occasions, however, it was far above this value, up to 20.4 lmg. But this sample is open to criticism because the analysis covers both the dissolved and the precipitated iron, only the former of which is dangerous to the fish (cp. pp. 8—9). As late as January 1948 the water proved to contain 12—12.5 lmg. of iron and, assuming that this sample was collected satisfactorily, the water must still be considered useless as a fishing water.

The content of sulphate not remarkable. The alkalinity seems to be somewhat fluctuating. An acidity was not established, which means that the acid reaction of the water is presumably caused by carbon dioxide alone. The pH value of 6.2 observed in January 1948 and the low alkalinity would seem to indicate a certain degree of acidity though hardly of any high size-order. The specific conductance not remarkable.

8. Conclusion; termination of the case.

It would be tempting to make a graphical comparison between the individual factors examined for all analyses and all localities of sampling in order to show their variation down the river; but for different reasons this cannot be done: the heterogeneity of the material (both with regard to localities of sampling and to the times at which the samples were collected) makes it difficult, and in certain cases the sampling itself is open to critism. A graphic representation must therefore be confined to one series of analyses, the most comprehensive one of all series—both as regards the localities of sampling and the factors examined—from April 30th, 1947 when, under due control, only samples of clear water were collected. The topographical variation of this series of analyses is shown in Fig. 8.

This diagram shows indisputably that

1. there is a radical chemical change of the Spaabæk water after the inflow of the drainage water from the area of the lignite pit;
2. its effect can be traced far down the river;
3. the Pølbæk—in spite of its more voluminous flow of water—does not predominate over the pathological water of the Spaabæk after the two brooks have flown together. On the contrary the Pølbæk—in spite of its not very excellent qualities—helps to mitigate the effect of the poisoned Spaabæk water;
4. there is a gradual improvement of the water of the Tim Aa in the direction of the Nørre Esp pond farm;
5. in the harmonic run of the curves for the river

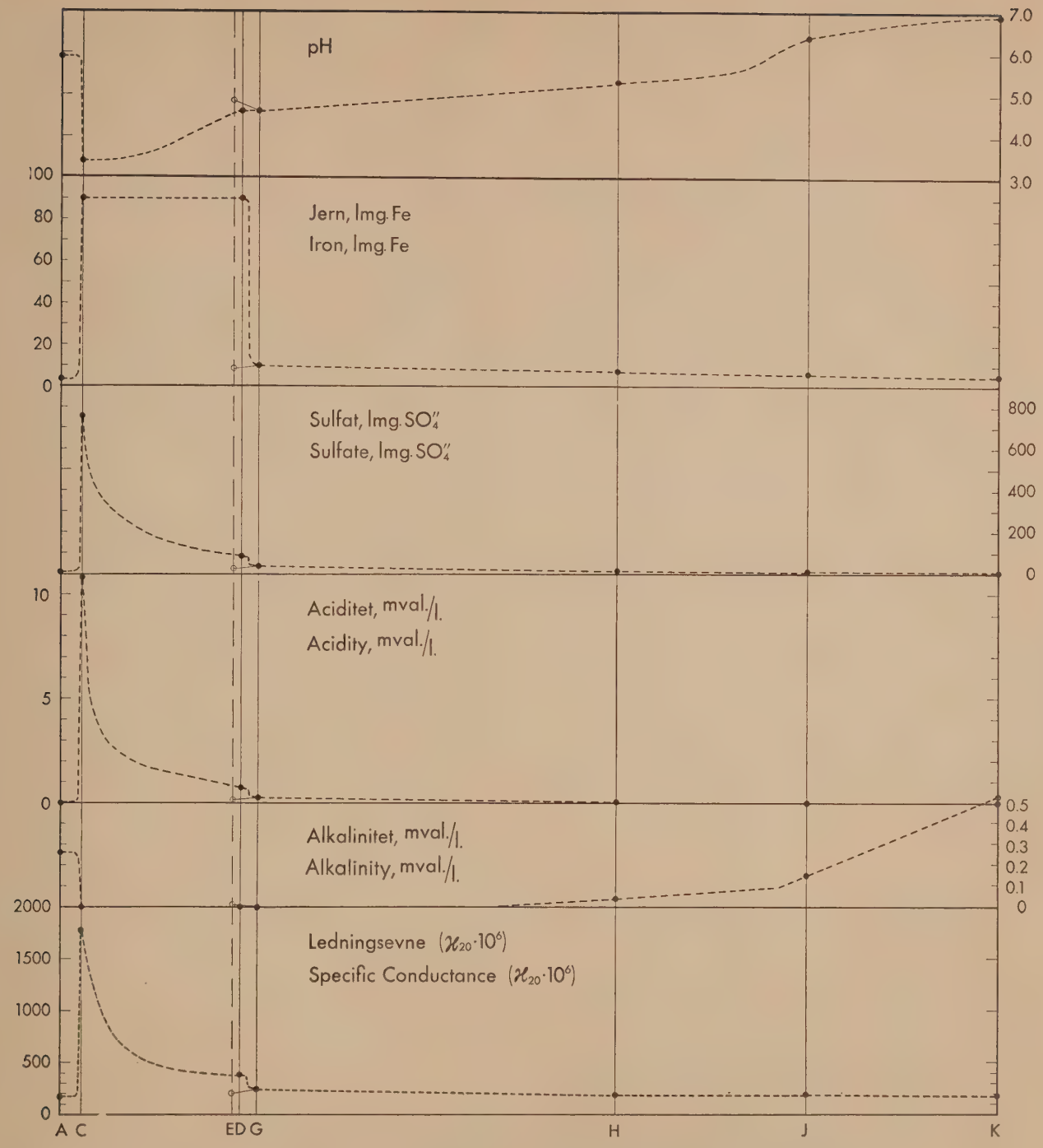


Fig. 8. Diagram showing the variation of the most important factors in some of the water samples from the Tim Aa area on 30-IV-47. Absciss: the localities of sampling at correct distances from each other (cp. Fig. 6, p. 15). The uneven run of the graphs for pH and alkalinity between stations H and I is explained by the supposition that the rise of the values occurred in these very localities (the occurrence of clay at Thorsted).

Tim Aa below the conflux of the two brooks there is no indication that water of a nature or a quantity that is able to effect the composition of the river water in a noxious way should be carried into the river from other sources than the brook Spaabæk.

In the face of these facts the pond farmers and the tenant of the lignite pit came to an extra-judicial agreement concerning the economic question and demanded the case to be withdrawn from the agricultural commission; this took place at the end of January 1948. According to the agreement the losses suffered by the pond farmers were made good, and furthermore the holders of the free water fishery were indemnified to some extent for the damage done to this fishery.

Irrespective of this compensation for losses suffered up to now the future fate of the Nørre Esp trout pond farm and the free water fishery in the river Tim Aa remains an open question. In the investigations discussed above there is nothing to indicate that the poisonous effects of the drainage water from the area of the lignite pit are decreasing. If no measures are taken to the effect of neutralizing and filtering the drainage water, we must face the fact that the pond farm and the river itself for perhaps a very long series of years may pass out of the production; to the fishery this is deplorable loss notwithstanding its background of necessity and the economical justification for the production of lignite on account of the war situation.

9. Definitions and methods of analysis of the samples from April 1947.

The reaction (the hydrogen-ion concentration, pH) was determined immediately after the reception of the samples; this was done with a pH-meter ("Rørjonometer", man. Radiometer) by means of glass electrode.

The specific conductance (the ion concentration) was determined immediately after the reception of the samples by means of bright platinum electrodes and measuring bridge "Philoscop" (man. Philips).

The iron was determined colorimetrically with potassium thiocyanate after previous treatment with potassium permanganate. These samples were not collected separately, but the determinations were made immediately after the reception of the samples.

The smell was examined in the series from $^{30}/_{4}$ 1947, partly from the cold water, partly after heating to about 60° C. in a covered Erlenmeyer flask; it was not remarkable in any case, and so no particular statements were made.

The sulphate was examined iodometrically by a method given by Høll (13). Double determinations were made in all cases. Filtering was carried out through glass filters (Jena 11 G 4). Qualitative sampling according to the "Danish Standard".

The alkalinity denotes the amount of acid to be added to the water in order to lower pH to 4.4, at which value practically all bicarbonates have been converted into carbonic acid.

The determinations were made electrometrically by means of the pH-meter by determination of the pH values resulting from rising amounts of acid. After adding of a surplus of acid and cold removal of the carbonic acid (see further under acidity) differential titrations with n/100 NaOH were made in the series from $^{30}/_{4}$ 1947 in order to establish the possible presence of other acid-binding matters than the bicarbonates; however, the maximum presence of such matters was found to be as low as 0.03 mval/l. No statements are therefore given in the tables, but the alkalinity is given as the total ability to bind acids; the content of bicarbonate, however, was calculated by differential titration.

In all cases the samples used were of 100 ml, taken with a certified pipette and n/100 hydrochloric acid with correction for the exact normality of the acid.

Corresponding to the term alkalinity the acidity stands for the amount of acid contained in the water.

The determinations were made electrometrically by adding rising quantities of sodium hydroxide (n/10 to the lignite water and n/100 to the other samples) with correction for the exact normality of the base.

The purpose is to determine how large an amount of base is necessary in order to neutralize the given sample. The determination was not started until a 30 minutes' cold removal of the free carbon dioxide present (aeration with air free from carbon dioxide) had taken place.

By means of a water blast apparatus the air is pressed through two glass flasks with 10—20 % potassium hydroxide, followed by a flask with soda lime (to absorb sprinkles); after this through a small control flask with destilled water, to which has been added some drops of sodium hydroxide and a few drops of phenolphtalein. When left standing the control is colourless, but colours red after aeration with air free from carbon dioxide, thus ensuring a satisfactory absorption in the first two flasks. The apparatus ends with an empty test tube, which is meant to catch possible sprinkles.

The titration itself was made in a cylindrical glass (about 200 ml) closed with a thick rubber stopper with borings (for the tip of the burette, the stirrer and the electrodes). The stirring is carried out by means of the air only, but is facilitated by now and then turning the feed tube, which is angular and pointed. A slit through the holes for the electrodes facilitates their application.

"Acids" are in these cases a collective concept for proper acids (weak and strong acids) and for other base-binding matters, more particularly ferrous sulphate.

10. Literature.

1. Schäperclaus, Wilhelm: Fischkrankheiten. 2. Auflage. Braunschweig 1941.
2. — Karpfenerkrankungen durch saures Wasser in Heiden und Moorgegenden. (Zeitschrift für Fischerei und deren Hilfswissenschaften, B. XXIV, 1926).
3. — Lehrbuch der Teichwirtschaft. Berlin 1933.
4. Otterstrøm, C. V.: Svovlsyreforgiftning af Vandløb fra udgrøftet Eng. Katastrofen i Ørreddambruget ved Lysbro. (Ferskvandsfiskeribladet, 36. Aarg. No. 1. 1938).
5. Christensen, Werner: Moseafvanding og Svovlsyreforgiftning i Vandløb. (Ferskvandsfiskeribladet, 39. Aarg. No. 6. 1941).
6. Pape, C. H.: Om Moseafvanding og Svovlsyreforgiftning i Vandløb. (Ferskvandsfiskeribladet, 39. Aarg. No. 8. 1941).
7. Czensny, Rudolf: Über die Löslichkeit von Eisenhydroxyden in Abhängigkeit vom pH-Wert. (Zeitschrift für Fischerei und deren Hilfswissenschaften, Bd. XL, 1942).
8. Ødum, Hilmar & Werner Christensen: Danske Grundvandstyper og deres geologiske Optræden. (Danmarks geologiske Undersøgelser, III Rk., No. 26, Copenhagen 1936).
9. Boisen Bennike, S. A.: Contributions to the Ecology and Biology of the Danish Fresh-water Leeches (Hirudinea). (Folia Limnologica Scandinavica, No. 2. 1943).
10. Olsen, Sigurd: Danish Charophyta. Chorological, Ecological and Biological Investigations. (Det kgl. danske Videnskabernes Selskab, Biologiske Skrifter, Bd. III, No. 1. 1944).
11. Iversen, Johs.: Studien über die pH-Verhältnisse dänischer Gewässer und ihren Einfluss auf die Hydrophyten-Vegetation. (Botanisk Tidsskrift, Bd. 40. 1929).
12. Wagler, Erich: Über die Systematik, die geographische Verbreitung und die Abhängigkeit der *Daphnia cuculata* Sars von physikalischen und chemischen Einflüssen des Milieus. (Intern. Rev. d. ges. Hydrobiol. u. Hydrographie, 11, 1923).
13. Höll, Karl: Oekologie der Peridineen. (Pflanzenforschung, H. 11, Jena, 1928).
14. Olsen, Sigurd & Knud Larsen: Er Furesøen ved at ændre Karakter? Et Bidrag til Diskussionen om Søens Eutrofiering. (Lystfiskeritidende, No. 467, July 1948).
15. Högbom, A. G.: Om vitriolbildning i naturen såsom orsak till massdöd av fisk i våra insjöar. (Svensk Fiskeri-Tidskrift, 30. årg. 1921).

11. Survey of all analyses of water from the Tim Aa area 1943–1948.

Date	Locality of sampling	Appearance of the water when sampled, etc.
/9 1943	Drain from the lignite pit	
—	The Tim Aa at Vinkælder	
—	The Tim Aa at the vicarage of Thorsted	
—	The Tim Aa at the dairy of Thorsted	
—	The Tim Aa at Sdr. Esp	
4/12 1943	The Tim Aa at the Nr. Esp pond farm	
7/3 1944	Drain of the dairy of Thorsted	Very dark
—	Drain of the lignite pit	Brownish
3/4 1944	The Tim Aa at the Nr. Esp pond farm	
—	Drain of the lignite pit	
—	The Spaabæk 300 m below the drain of the lignite pit	
—	The Tim Aa at Thorsted	
17/12 1945	Drain from the pumping pipe of the lignite pit	Clear, colourless
—	Draining ditch abt. 50 m above its fall into the Spaabæk	Greyish-brown, turbid
—	The Spaabæk 25 m above the drain of the pit	Greyish, turbid
—	The Spaabæk 40 m below the drain of the pit	Greyish, turbid
—	The Spaabæk 125 m above its confluence with the Pølbæk	Greyish, turbid
—	The Pølbæk 75 m above its confluence with the Spaabæk	Turbid
—	The Tim Aa 25 m below the confluence of the brooks Spaabæk and Pølbæk	Somewhat purer than the previous
—	The Tim Aa at Vinkælder	Brownish
—	The Tim Aa at the dairy of Thorsted	More brownish than the previous
—	The Tim Aa at the Nr. Esp pond farm	Still more brownish. Slightly turbid, colourless
—	Drain of the dairy of Thorsted	Water nearly stagnant
22/2 1946	Drain of the lignite pit	Clear (no pumping from the pit)
—	Drain of the lignite pit	Turbid (full pumping from the pit)
24(?)/2 1946	Drain of the lignite pit	Clear (no pumping from the pit)
—	Drain of the lignite pit	Turbid (full pumping from the pit)
11/4 1946	Drain from the pumps of the lignite pit	
—	The Spaabæk above the drain of the lignite pit	
—	The Spaabæk 125 m above its confluence with the Pølbæk	
—	The Pølbæk 75 m above its confluence with the Spaabæk	
6/9 1946	Draining ditch above its fall into the Spaabæk	Clear
—	The Spaabæk abt. 25 m above the drain of the lignite pit	Somewhat turbid
—	The Spaabæk abt. 125 m above its fall into the Tim Aa	Turbid, partially from suspended ochre
—	The Pølbæk 75 m above its confluence with the Spaabæk	Brown from suspended ochre
—	Affluent to the Tim Aa from the north through the easternmost drain at Ab isgade	Clear. Roughly 5—6 l/sec.
—	The Tim Aa at the Nr. Esp pond farm	Clear, but with a few small particles (ochre ?)
21/4 1947	The Spaabæk above the drain of the lignite pit	
—	Drain from the overburden of the lignite pit	
—	The Spaabæk below the drain of the lignite pit	
—	The lower end of the Pølbæk	
—	The Tim Aa at the Nr. Esp pond farm	
30/4 1947	The Spaabæk above the drain of the lignite pit	Slightly opalescent
—	Drain from the overburden of the lignite pit	Clear
—	Lower end of draining ditch of the lignite pit	Clear
—	The Spaabæk below the drain of the lignite pit	Opalescent, milky
—	The lower end of the Pølbæk	Opalescent, slightly milky
—	Ochre ditch falling into the Pølbæk	Clear
—	The Tim Aa abt. 100 m below the confluence of the Spaabæk and the Pølbæk	Opalescent, slightly milky
—	The Tim Aa at Vinkælder	Slightly opalescent
—	The Tim Aa at the dairy of Thorsted	Opalescent
—	The Tim Aa at the Nr. Esp pond farm	Slightly opalescent
2/1 1948	The Tim Aa at the Nr. Esp pond farm	
/1 1948	The Tim Aa at the Nr. Esp pond farm	

[1]) DBS: The Danish Biological Station. FF: The experimental Laboratory of the Board of Fisheries. Q: Qvist's Laboratory, Aarhus. Sig. O.: Sigurd Olsen.

Reaction pH	Iron mg Fe	Sulphate mg SO_4^{--}	Alkalinity mval/l	Acidity mval/l	Bicarbonate mg HCO_3^-	Hardness °DH Total	Hardness °DH Carbonate	Specific conductance $\varkappa_{20} \cdot 10^6$	Consumpt. of oxygen w. KM_nO_4 mg oxygen	Analysed by[1])
5.4	..	..	..	..	..	6.0	..	..	..	DBS
5.8	..	..	..	..	..	2.5	..	..	..	—
5.8	..	..	..	..	..	2.0	..	..	..	—
6.4	..	..	..	..	..	2.0	..	..	..	—
6.8	..	..	..	..	..	3.0	..	..	..	—
6.7	2	..	..	..	..	..	..	..	4.4	FF
5.4	8	..	..	..	..	..	..	..	327	—
5.7	3	..	..	..	..	..	..	..	75	—
6.6—6.8	19	..	..	..	..	..	..	..	..	—
4.2	131	..	..	..	..	..	..	..	..	—
5.4	..	..	..	..	..	..	..	..	..	DBS
5.8—6.0	..	..	..	..	..	..	..	..	..	—
4.17	260	1461	..	..	..	..	..	..	..	Q
3.77	260	1460	..	..	..	..	..	..	3.8	—
5.68	1.3	27	..	..	..	..	..	..	7.3	—
4.68	12.2	90	..	..	..	..	..	..	6.0	—
4.47	5.1	80	..	..	..	..	..	..	4.4	—
4.74	6.5	44	..	..	..	..	..	..	5.8	—
4.89	3.8	59	..	..	..	..	..	..	4.1	—
4.75	13.3	..	..	..	..	..	..	..	..	—
5.01	19.4	..	..	..	..	..	..	..	..	—
5.97	20.4	..	..	..	..	..	..	..	..	—
6.55	1.4	..	..	..	..	..	..	..	..	—
3.72	165	..	..	..	..	..	..	..	..	FF
3.51	255	..	..	..	..	..	..	..	..	—
4.12	250	1274	..	..	..	..	..	..	..	Q
3.95	260	1376	..	..	..	..	..	..	..	—
4.12	110	..	..	..	..	..	..	..	..	FF
7.18	3.5	..	..	..	..	..	..	..	..	—
4.35	52.5	..	..	..	..	..	..	..	..	—
7.15	3.5	..	..	..	..	..	..	..	..	—
3.00	66	..	..	..	..	..	..	..	..	—
5.90	12	..	..	..	..	..	..	..	..	—
3.55	14	..	..	..	..	..	..	..	..	—
4.80	24	..	..	..	..	..	..	..	..	—
6.25	8	..	..	..	..	..	..	..	..	—
7.0	11	..	..	..	..	..	..	..	..	—
6.1	1.5	+	0.22	..	13	..	0.6	173	..	Sig. O.
3.1	200	+++	0	21.2	0	..	0	2600—3100	..	—
3.4	12	+	0	0.85	0	..	0	520	..	—
4.4	4	+	0	0.15	0	..	0	226	..	—
6.6	1.5	+	0.52	..	32	..	1.5	191	..	—
5.9	3.5	4.6	0.26	0	14	..	0.7	173	..	—
3.4	175	1509	0	20.9	0	..	0	2655	..	—
3.4	90	756	0	10.8	0	..	0	1785	..	—
4.6	90	85	0	0.71	0	..	0	381	..	—
4.8	8.5	23	ca. 0.01	0.13	ca. 0.6	..	+	202	..	—
6.1	3.1	1.4	0.13	0.02	8	..	+	121	..	—
4.6	10	39	0	0.25	0	..	0	241	..	—
5.3	7.5	17	0.04	0.04	2.5	..	+	191	..	—
6.4	6.2	13	0.15	0	8	..	0.4	186	..	—
6.9	4.7	7.1	0.53	0	31	..	1.5	181	..	—
6.8	12	..	0.3	..	..	..	..	..	..	FF
6.2	12.5	..	ca. 0.1	..	..	..	..	..	..	—

On a Recent and Frequently Occurring Tumor Disease in Eel.

By

M. CHRISTIANSEN and AAGE J. C. JENSEN.

CONTENTS

Page

Introduction 31

I. Aage J. C. Jensen:

Occurrence of the Disease in the Various Years and its Distribution 32
Beginning of the Disease 32
Distribution and Frequency of the Disease 32
Occurrence of the Disease in Yellow and Silver Eels 34
Size of the Eels Affected 35
Locality of the Origin of the Disease 35
Addendum 37

II. M. Christiansen:

Investigations of the Tumors 38
Occurrence and Macroscopic Appearance 38
Histologic Examination 42
Attempts at Transmission 44

Introduction.

In October 1944 the Danish Biological Station received a silver eel of 53 cm., caught near the southern point of Amager, which round its mouth had one of the tumors to be discussed below. In the autumn of 1945 the Station received another, which was examined at the Pathologic-Anatomical Laboratory of the Royal Veterinary and Agricultural College. In 1946 a number of such eels were also sent to the State Veterinary Serum Laboratory (Professor M. Christiansen), and as the fishermen from some areas complained that the disease was so frequent that it was detrimental to the fisheries because the diseased eels were unsaleable, a collaboration between the present writers was introduced in order to investigate the occurrence and cause of the disease.

By request the staff of the Fisheries Control reported on the occurrence of the disease to the Ministry in Dec. 1946, Jan. and Feb. 1947, and in Jan. 1948, and by enquiries to the chairmen of a number of fishermen's associations and to other experts on fishing in April 1947 and the spring of 1948 we received further information.

We offer our best thanks to the great number of persons who have readily given their assistance by offering information. Some of them will be listed below. In the description of the occurrence of the disease there are references to the sources from which the information originates, the number of the informant in question in the subjoined list being added.

List of Informants.

1. Dr. Bogucki, Gdynia.
2. Professor T. H. Järvi, Helsingfors.
3. Dr. Chr. Hessle, Stockholm.
4. Dr. N. Rosén, Gothenburg.
5. Dr. G. Svärdson, Drottningholm.

The following chairmen of fishermen's associations: 6. L. Holberg Andersen, Grenaa. 7. Carl H. Bloch, Horsens. 8. Otto Boesen, Nykøbing F. 9. Th. Burmann, Bisserup. 10. Chr. L. Christensen, Struer. 11. Georg Christensen, Sønderborg. 12. Sv. Aa. Christensen, Sundstrup. 13. Hans Dvinge, Vejle. 14. Hans Hansen, Skagen. 15. Niels Hansen, Nyord. 16. Viggo Hansen, Kolding. 17. Jens Jensen, Drejø. 18. Ludvig Jensen, Rønne. 19. H. A. Jacobsen, Holbæk. 20. A. Johansen, Vigerø. 21. Carl Johansen, Dyreborg. 22. Vilh. Johannesen, Randers. 23. P. Lahm, Langøre. 24. Holger Larsen, Thisted. 25. Hans Martinussen, Kerteminde. 26. Hans Møller, Sebbersund. 27. Holger Nielsen, Langø. 28. Jens Chr. Nielsen, Hvalpsund. 29. Nis Nielsen, Egernsund. 30. Peter Nielsen, Reersø. 31. P. Petersen, Karrebæk. 32. O. Sommer, Køge. 33. A. Strandby, Aabenraa. 34. Chr. Sørensen, Taars. 35. A. Vallentin, Knebel.

Further: 36. Mateo Andersen, Manager to the Fishermen's Cooperative Sales Association, Holbæk. 37. N. C. Christensen, Typographer, Copenhagen. 38. Poul Christensen, Fisherman, Thyborøn. 39. Th. Heintzelmann, Shipmaster, Læsø. 40. H. Holm Hansen, Fishery-Assistant, Lellinge. 41. Fr. Jensen, Fishing Lessée, Engestofte, Maribo. 42. W. Kromann, Harbourmaster, Bagenkop. 43. A. Larsen, Manager to the Fishermen's Cooperative Sales Association, Assens. 44. Martin Nielsen, Fisherman, Copenhagen. 45. Johs. Pedersen, Postman, Jebjerg. 46. Herman Thorsen, Fisherman, Tejn. And

The Ministry of Fisheries (particularly Mr. Strubberg, Head Clerk) and 47. The staff of the Fisheries Control.

Further, thanks are due to Mr. Vilhelm Jensen, Fish Exporter, Roskilde, who has placed many healthy eels at our disposal for the experiments free of charge.

Many of the experiments were made in the Biological Aquarium, where aquaria were kindly placed at our disposal.

I. Occurrence of the Disease in the Various Years and its Distribution.

By Aage J. C. Jensen.

As mentioned above the distribution and occurrence of the disease has chiefly been ascertained by information received from fishermen and other experts on fishing, and as in eels various tumors of a different kind from that described here may be found, we should of course be cautious as regards statements of occurrence which it has proved impossible to check. Amongst others in cases where the occurrence was remarkable, we have therefore asked for further information and the informant in question has been shown a drawing or photograph of eels affected by the disease.

It may be mentioned that information was received from the Isefjord that tumors had been observed on yellow eels in the spring of 1947 (47). From an interview with the informant (36), who gave an excellent description of the symptoms of the disease observed on the Isefjord, it appeared that they were the nodes which may develop on eels as a consequence of the cold, while the tumors described here according to statements by our informant and several other persons are not known at all from the Isefjord.

From Thyborøn information was received that in October 1947 two silver eels with wart-like tumors had been caught (47). By an interview with one of the fishermen (38) it was learnt that the latter had later heard of several other cases of the same disease, but from the description of the disease it seems established that this was a case of eels which have been severely attacked by red-disease. This disease not rarely ravages the Limfjord badly, and it may be added that in a report from Hvalpsund that eels with the cauliflower-like tumors are unknown there, it has been expressly stated that eels with red-disease may have large cyst-like tumours on abdomen and sides (28).

Beginning of the Disease.

As appears from the introduction the disease has been of an epidemic character. Some fishermen from the areas south of Zealand have informed the Fisheries Control that the disease was observed also before 1944, but so rarely that it had aroused no attention, and we have obtained the same information direct from some fishermen from the same area. From repeated enquiries to the latter it seems, however, in most cases to have been another kind of tumors. Some fishermen from Karrebæk, however, state that in their opinion the disease also occurred several years ago (31). From the same areas others state that the disease has not been observed until 1945 or 1946, which clearly shows that the disease, if it occurred before 1944 at all, at any rate was very rare.

From the south and west coasts of Skåne, Bornholm, and the east coast of Zealand (Køge) it is stated that the disease was not observed until 1944 and that it has spread later (3, 4, 32, 47). In 1945 a few affected eels were caught at the south coast of Møen, the east coast of Falster, and the south coast of Lolland, and from Blekinge there are also reports on findings since that year. In the other areas the disease has not been observed until 1946, at the east coast of South Jutland, in the Bay of Aarhus even in that year only (47), and at Västervik, N. of Kalmarsund, it was not observed before 1947 (3).

Distribution and Frequency of the Disease.

According to all information received the disease has never been observed in freshwater,—neither in Denmark, Sweden, nor Poland (1, 3, 40, 41, 44, 46, 47, etc.).

It is known from the southernmost Sweden, Poland, Bornholm, the east coasts of Zealand and Møen-Falster, the Western Baltic, the Belt Sea (except the coast facing north) and from a single finding near the Skaw. According to information received from Professor Järvi it is unknown from the seas of Finland.

While the disease in 1944 was only observed in a few sporadic cases at South Sweden, Bornholm, the east coast of Zealand, and probably also at West Lolland, some cases are reported from Køge Bay and Fakse Bay in 1945, and further it is reported that at the south coast of Møen, the east coast of Falster, and the south coast of Lolland on an average 1 per thousand of the silver eels caught was affected by the disease (47); in some of these areas, however, f. inst. S. of Møen, the disease was observed more rarely. In 1945 it was first noticed at West Lolland (27). It is unknown of how frequent occurrence it has been at the east coast of Zealand.

The map in fig. 1 schematically shows the distribution in 1946.

In 1946 the number of diseased eels increased in all areas except along the south coast of Møen-Falster-Lolland, where it remained comparatively unchanged (47). The chairman of the Fishermen's Association of Nykøbing F. states that at the part of the Baltic coast of Falster included in the area of the Association a total of about 40 silver eels with tumors was caught (8). This is probably somewhat below 1 per thousand. The disease was most frequent at Poland, where about 1 per cent. of the eels was affected, and along the south coast of Sweden (the south and east of Skåne, and Blekinge), where, however, less than 1 per cent. of the eels seems to have been affected by tumors. Along the the east coast of Zealand it was also comparatively common. At Køge the number of diseased silver eels was estimated at about 3 per thousand (32). In a few other places also a fairly good number might be found, thus in the Lesser Belt (Lillebælt, at Frydensborg and Fænø) up to 10 specimens in 100 kilos (47), which is nearly 2 per cent. of the number. At

Fig. 1. Schematic map of the distribution of the disease in 1946. The width of the black line along the coasts indicates approximate relations between the frequencies in the various places. The figures on the map refer to the numbers of some of the informants mentioned in the Introduction (p. 29).

the north coast of the Smaaland Sea probably only somewhat more than 50 specimens were caught (47). Along the west coast of Zealand (Sjælland), there were in two catches of in all about 6 000 kilos about 30 diseased eels, i. e. about 1 per thousand, but none in most of the other catches (30).

In all the other areas in which the disease was observed there were only a few diseased eels (see the map).

In 1947 the disease seems to have decreased along the south and east coast of Skåne (3,4), apart from an extraordinarily large number of cases of yellow eels caught at Trelleborg, it being stated in April 1948 that 10—15 per cent. of yellow eels caught were affected (see below on the occurrence in yellow and silver eels).

Also at Blekinge the disease seems to have kept unchanged and also on an average to have been observed in less than 1 per cent. of the eels. From Åhus in the bay between Skåne and Blekinge it has been stated by J. W. Nilsson, eel exporter, that among 3 million eels there were 3—4 silver eels and no yellow eels with cauliflower-like tumors (4). Towards the north the disease spread through Kalmarsund, a few affected silver eels having been caught at Västervik (3).

Along the east coast of Zealand the disease kept nearly unchanged; in some fishermen's opinion, however, it had also abated here. It has been estimated that about 5 per thousand of the silver eels in Køge Bay and Fakse Bay were affected in 1947, in the Sound north of Copenhagen at some places only 1 per thousand (47). At Landskrona the number was estimated at 5 per thousand, at Höganäs even 2—3 per cent., while from Mölle only a few cases have been reported (4). Along the south coast of Møen, the east coast of Falster and the south coast of Lolland the frequency also remained unchanged, about 1 per thousand (47). At West Lolland and Bagenkop there were somewhat fewer cases in 1947 than in 1946 (34, 42).

Occurrence of the Disease in Yellow and Silver Eels.

Yellow eels with tumors have particularly been frequent near Poland, where according to Dr. Bogucki they amounted to 1 per cent. of the eels caught in 1947.

On good authority it may be stated that there were many of these eels among the yellow eels which in 1945, 1946, and 1947 were caught off North-East Bornholm (46). According to another good authority they were particularly observed near Bornholm in June-July-August 1947, although in few cases (47). The terms "many" and "few" are of course very relative and may very well cover each other. At Bornholm few silver eels are caught, and chiefly off North-East Bornholm. Tumors have not been observed in these by one of the sources mentioned above (46), who carries on a fairly extensive eel fishing, but from other quarters it has been reported that probably a few silver eels with tumors were caught in 1946 (47). A yellow eel with a typical cauliflower-like tumor near its mouth in the autumn of 1946 was sent to the Biological Station from Bornholm.

From the south coast of Sweden it has been reported that the tumors have also been observed in yellow eels (3). Manfred Johansson, eel-fisher and fisheries surveyor, reports from the east coast of Skåne (4) that he has never seen affected yellow eels; hence they must be extremely rare along this coastline. But H. Werner Hansson, Manager of the Cooperative Association of the Malmöhus Län Eelers, in the spring of 1948 reported from Trelleborg that the disease in the beginning (in 1944 or 1945) occurred only in a few silver eels, but that it had now attacked the yellow eel, too, which is considered still more susceptible to the disease. According to statements by fishermen within the district it was considered that 10—15 per cent. of the yellow eels were affected, while among the silver eels few diseased eels were found (4). From Ålaboderna, N. of Landskrona, Johan A. Jönsson, fisherman and fisheries surveyor, reports that in the autumn he caught 10 000 kilos silver eels and 400 kilos yellow eels, and that tumors were found in 50 kilos silver eels but in no yellow eels (4).

In the Danish waters, apart from those round Bornholm, the disease in the great majority of cases has been observed in silver eels. Still, yellow eels with tumors have also been caught in some places here.

It was reported that a few yellow eels with tumors were caught in Præstø Fjord in the early spring of 1946 (47), but these tumors are hardly due to the disease discussed here. From the autumn of 1947, on the other hand, there are reports on a few catches of yellow eels with cauliflower-like tumors from the east coast of Zealand (47), and a few of these eels

were sent to Professor Christiansen for examination. Further a few yellow eels with such tumors were found at the south and west coasts of Zealand, in the fjords at Karrebæksminde about 40 out of a total of about 250 000, or barely 0.2 per thousand (47). From the waters south of Funen there is a report of a single yellow eel with a tumor, which in the summer of 1947 was caught near Drejø (47). From other quarters it has been reported that no diseased eels were caught before September (the beginning of the season of the silver eel fishery), but then there were many (17).

Size of the Eels Affected.

The affected eels caught at Bornholm were small, below about 250 grammes (46), chiefly below 125 grammes. In the Karrebæksminde fjords they were of 100—250 grammes (47), i. e. of nearly the same size as those not diseased.

All sizes of silver eels may be affected, but along the south coast of Sweden the disease has been most frequent in the minor eels, up to ½ kilo (3,4). From the bays of Køge and Fakse the sizes of the affected silver eels were reported to be about ½—1 kilo in 1946 (32), in 1947 about ½ kilo (47), at Vordingborg about ½ kilo and upwards (47).

At South-West and West Zealand the sizes of the affected silver eels were estimated to be 250 grammes and upwards (31), 375—500 grammes (9) or 300—500 grammes (30), in 1947 250 grammes—1 kilo, while the average of all silver eels caught was here about 175 grammes each (47).

As the male silver eels rarely weigh more than 150 grammes, each, extremely rarely more than 160 grammes, it is seen that there must have been very few male eels among the affected silver eels. This also agrees with the fact that the majority of affected silver eels received for examination were more than 45 cm. in length, thus being mainly female eels.

Locality of the Origin of the Disease.

As the silver eels caught in the autumn are migrating towards more saline water, towards the Atlantic, the occurrence of silver eels caught in some locality shows that the disease must have been contracted either in this place or somewhat farther in the pirection of the more brackish water. The fact that the disease has not been observed in freshwater either in Denmark, Poland, or Sweden is fairly good evidence that it does not originate from freshwater.

The distribution of the occurrence of the disease, as shown in fig. 1, seems to indicate that the chief hotbed of the disease is somewhere in the eastern Baltic. Along the east coast of Zealand (Sjælland), the south coast of Møen, and the east coast of Falster the eels caught are chiefly migrating as silver eels from the Baltic towards the ocean. It appears from the map that also the other places where diseased silver eels have been caught are localities where it may be expected that the silver eels from the Baltic migrating towards the ocean may strike the coasts, while the diseased eels have not been found in localities sheltered from this migration, e. g. west of Møen (15), the north coast of Lolland, or the archipelago south of Funen (Fyen) (17, 47) and the Isefjord (36).

The fact that the great majority of the diseased eels are females agrees with the view that the disease is contracted somewhere in the inner Baltic, because, as is well-known, there are very few male eels to be found east of Bornholm. The fact that the sizes of the diseased silver eels seem to be somewhat smaller near South and West Zealand than near East Zealand perhaps may be due to the emigrating diseased female eels, which come from the Baltic, in the Belt Sea being mixed with both males and females that had contracted the disease in our waters.

The yellow eels do not by far migrate so much. As far as our knowledge goes, their migrations are partly a slow migration of the young females towards more brackish water, partly migrations to and fro between the quite shallow waters along the coasts with grass or a dense growth of seaweed, where most of the yellow eels feed in summer, and the somewhat deeper water with a soft bottom, in which many of them hide in winter (as is well-known, many dig themselves into the bottom at a depth of a few metres).

The fact that the yellow eels in our waters may have tumors therefore seems to be a proof that the disease in some cases may be caught in our waters.

The comparatively frequent occurrence of yellow eels with this disease near Poland shows that any rate one of the hotbeds of the disease is found there. Unfortunately political conditions makes it impossible at present to obtain information about the distribution of the disease along the other southern and south-eastern coasts of the Baltic (as mentioned

above the disease has not been reported from Finnish waters). The still higher percentage frequency of the disease in yellow eels near Trelleborg (where yellow eels, however, are comparatively few in number) shows that undoubtedly also other localities may be considered as principal hotbeds of the disease.

It cannot yet be decided whether the disease may be caught by eels at the silvery stage. The great number of unsuccessful attempts at infecting healthy silver eels by making them swim with diseased ones do not tell much about this as it has not been possible to infect healthy yellow eels in this way either, nor do they tell that the disease is not infectious. Perhaps a certain particularly high or particularly low temperature is necessary for contagion, perhaps some other factor in the experiments has been unfavourable for contagion.

Professor Christiansen's observation of the fact that the tumors may spread on an affected silver eel would seem to indicate that silver eels may also be infected from outside.

The fact that the disease is generally seated at the mouth of the eels[1]), suggests that it is produced by something eaten by the eels, and if so, of course only yellow and silver eels at the beginning of this stage could contract the disease, but the occurrence of the tumors on the head might also be due to the disease being caught by the eel digging itself into the bottom, and if so, yellow and silver eels of course would be nearly equally exposed to infection. If the disease is not due to infection, but is produced by a mechanical or chemical action on the skin, this cause must be particularly frequent in the Polish area and near Bornholm and South Skåne. The occurrence of yellow eels with tumors in some places in the Danish waters (particularly the Karrebæksminde fjords), however, seems to show that the same cause may also occur in these places, and then it is difficult to guess at the cause.

If the disease is an infectious disease there may be a possibility that it is infectious only at certain conditions of salinity or temperature. The fact that it has not been observed in freshwater would seem to indicate that a certain salinity is necessary for infection. The experiments show that normally the tumors do not disappear in freshwater, but that on the contrary it may develop further in freshwater on an eel that has already been affected by the disease.

As the Polish coast is one of the hotbeds of the disease it is reasonable to suppose that a physical factor characteristic of this area favours the disease.

The salinity in the spring is about 5 per thousand, in the autumn and the winter about 7 per thousand, and the variations from year to year amount to at most about 1 per thousand above or below these values. Quite similar conditions of salinity are found near the Swedish coast in the Kalmar area, where the disease has been of much rarer occurrence than at the adjoining more southerly coasts of Sweden. For instance in the Danish fjords we have also in many places similar salinities.

The temperature in winter, which on an average is a little below 1° C. in the monthly mean of February, is not characteristic of the area, either, but the same winter temperature is found along all the coasts of the southern and middle Baltic and the Belt Sea.

It should, however, be mentioned that it has been observed that both yellow and silver eels kept in well-boxes (corfs) in Kolding developed blueish-red cauliflower-like tumors and died soon after, as the water became too little saline in the winter of 1946/47. Because of the ice the well-boxes (corfs) could not be moved to more saline water (16). It has not been possible to ascertain whether these tumours were of the same kind as those which are being investigated here, but the description agrees with them, and in this connexion we may remind of the fact that the case mentioned by Professor Christiansen (p. 39), in which the tumours spread very much, occurred in an eel which in the winter of 1947 was kept in an open-air basin with freshwater in which the temperature was kept a little above freezing-point.

The summer temperature in the Polish area, on the other hand, is exceptionally high, the monthly mean of August being above 17° C., which high mean temperature besides is found only in the innermost part of the Gulf of Finland (where eels are comparatively few in number), and in the western Baltic[2]). An equally high mean temperature, however, may be found in other Danish coastal waters.

[1]) Manfred Johansson (the east coast of Skåne) states that he has never seen tumors in any places but the head, in 90 per cent. of the cases above the mouth (4).

[2]) Deutsche Seewarte: Atlas für Temperatur, Salzgehalt und Dichte der Nord- und Ostsee. Hamburg 1927.

As the climate near Poland is more continental in character than that of the entrance to the Baltic, the fluctuations from year to year undoubtedly are highest near Poland. It is worth noticing that the summer temperature in some recent years has just been extraordinarily high, viz. in 1932, 1937, 1938, 1941, 1944, and 1947. Before 1932 we must go right back to 1914 for a summer temperature as high as that of these years. As mentioned on p. 30 the disease began to become more frequent in 1944, and the great number of yellow eels with tumors near Poland were observed in the autumn of 1947 and yellow eels with these tumours have been much more frequent in Danish waters in 1947 than in 1945 and 1946.

Addendum.

After the Danish edition had been printed we have been informed by Mr. H. Werner Hansson that nearly all the small yellow eel caught at Trelleborg in the first part of the summer 1948, were affected by the disease. Later in the summer the number of affected yellow eel was somewhat lower, and of the silver eel only about 1 per cent. was affected along the whole coast of Sweden.

Some of the yellow eels caught were transplanted to Swedish lakes, and on this occasion it was impossible to sort off all the diseased eels, but in no case was the disease observed later on in the lakes to which the eels had been transplanted.

II. Investigations of the Tumors.

By M. Christiansen.

Occurrence and Macroscopic Appearance.

In some of the places mentioned in the foregoing section, eels affected with tumors have been collected, most of them by the Fisheries Control and others by Mr. N. C. Christensen of Copenhagen. These eels, which were sent to us for further examination, were for a shorter or longer time kept in aquaria, where they could be under constant observation. Some of them were dead, however, when received, or died shortly after. Especially some which were received early in the spring of 1947 and which had been kept in fish ponds in an eel store during the winter showed very little vitality and soon died in the aquaria, as a rule much affected with saprolegnacea and probably weakened by having been kept so long in store. A number of eels received in the fall of the same year, immediately or only a few days after they had been caught, kept alive much better. Some of these came from the "Kalveboderne" and the Sound, and one from the Great Belt, off Skelskør; but most of them from the Baltic, off Stevns. As for those which we received from the eel store, it cannot be said precisely where they had been caught.

Nearly all the eels were silver eels. They were of various sizes, from 30 to 70 cm. long, but most of them rather large (60—70 cm.). Two of those with tumors were typical yellow eels, respectively 36 and 40 cm. long, and one eel, about 40 cm. long,was in the transitional stage between yellow and silver eel.

The tumors vary in size and extent, but were in all cases very conspicuous. Of course it is especially the more obvious cases that have attracted attention and have been sorted out to be sent to us; tumors in the initial stage will probably easily be overlooked, nor were there any specimens of such among the material received. Though the growths, as said, may vary as to size, extent and location, they are all of exactly the same type. They occur only in the skin, and have not been observed either in mucous membranes or in internal organs. Their base is broad, sharply delimited from the surrounding, normal skin. In shape they are roundish, oftenest very prominent, the larger ones projecting as much as 1 to 2 centimeters. More rarely the more extensive growths present a somewhat flattened, not so prominent appearance. The surface is rather uneven, slightly rough or knobby, and between these lesser projections there are usually deep, but very narrow fissiform indentations or clefts, which as a rule are not seen, however, until the tumor is cut through, because their walls lie close up against each other. The appearance of the tumor may best be described as resembling that of a diminutive cauliflower. The color varies from light reddish grey to a deeper, fleshy red; in a couple of cases it has been almost bluish grey or slaty, owing to strong pigmentation. The consistence is soft, elastic; and even very slight traumas easily result in superficial, bleeding wounds. As the histologic examination shows, the connective tissue stroma is fairly well supplied with blood vessels, as might also be expected from the reddish coloring of the tumor tissue.

In nearly all the cases, the tumors have been situated on the head, and besides there may then be a greater or lesser number of tumors on various parts of the body and fins. But the tumor may also be confined to the head alone, especially to its extreme anterior part. This was the case, for instance, in the two yellow eels examined. In these, this whole portion, from the tip of the snout to the eyes, as well as the whole anterior part of the lower jaw, were the seat of very prominent, bright red tumor masses, which, when the mouth was closed, formed a single, roundish

tumor as large as a good-sized hazelnut (Fig. 1). But in a number of the eels examined, the tumor formations had assumed much greater proportions,

Fig. 1. Yellow eel with tumors on upper and lower jaws; the mouth open. Natural size. (M. Christiansen phot.).

extending over the whole of the head and the anterior part of the body. As Fig. 2 shows, the appearance may become quite grotesque, the whole head, including the eyes and the mouth, being enveloped in large, thick, cauliflower-like masses of tumor.

Fig. 2. Silver eel, viewed from above. The head and the anterior part of the body covered by a single, cauliflower-like tumor. $\frac{4}{5}$ natural size. (M. Christiansen phot.).

When these masses are cut thorugh, in to the eyes, it is found that neither the cornea nor any of the interior parts of the eyes themselves are engaged; the tumor growth arises solely from the skin, but also involves the latter right up to the margin of the cornea. The tumor masses thus grow together over the eyes, covering them with layers of tumor tissue, sometimes more than a centimeter thick. In the same way, also the aperture of the mouth is entirely surrounded by enormous masses of tumor, which cover the skin right up to the edge of the oral mucosa. The latter is not involved, but the parts of the tumor nearest it bulge in over it somewhat. When the mouth is closed, it is entirely covered by the surrounding masses of tumor and is only indicated by a very narrow slit; nevertheless it can be opened fairly wide. In some of the eels, the large tumor formations have occurred only on the upper aspect of the head, that is to say on the snout, the upper jaw and the occipitofacial parts, sometimes also on the anterior, upper part of the body; while the lower jaw and the

Fig. 3. Silver eel with knobby tumors on the upper jaw and occipitofacial parts of the head, smaller tumors on the back and the right pectoral fin. $\frac{5}{6}$ natural size. (M. Christiansen phot.).

ventral portions of the body have been either entirely free or only the seat of very small tumors (Fig. 3).

Together with tumors on the head there are, as said, often tumors on the body and fins; but there they usually only occur dispersedly and as a rule do not, each of them, become anywhere nearly as large as it may be the case with those on the head. They may be of very different size in the same eel, for instance from pinhead-sized to as large as a walnut; besides, they are often flattish, not as much projecting as those on the head. On the fins, where they are sometimes situated away out on the free edge, they may be almost disklike, compressed. As a rule these tumors on the body are found in much greater numbers on the dark portions of the skin than on the whitish, ventral side.

In a few cases, also tumors on the body have been very numerous and extensive. A particularly notable instance of this was a silver eel, about 70 cm. long, caught in the Great Belt, whose appearance will be seen from Fig. 4. Besides enormous tumor

formations over the whole of the head, it had on nearly every part of the body and fins great masses of roundish, partly confluent, reddish grey tumors, from quite small to the size of peas; and as the photograph shows there were a great many of these also on the lighter, ventral side of the body.

extending down over the latter,—in other words on the pigmented skin. In one case the tumor measured 4.5 cm. in diameter. In two of the cases there was only this one tumor, the other two had, besides, some few, scattered tumors on the pigmented skin of the body.

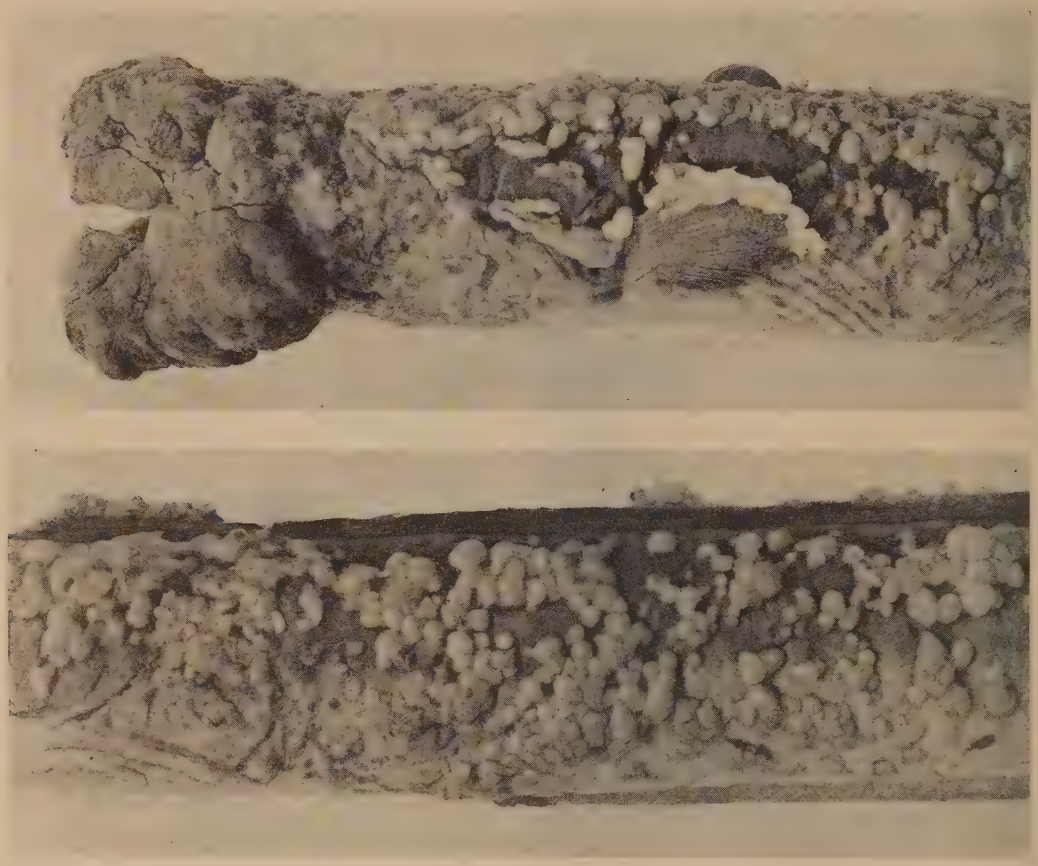


Fig. 4. Silver eel with large, very prominent masses of tumor covering the head. The slit anteriorly is the aperture of the mouth. Also an enormous crop of tumors all over the body, and a line of confluent tumors on the dorsal side of the left pectoral fin. The lower figure shows a portion of the body nearer the tail, the region about the anus. Natural size. (M. Christiansen phot.).

Fig. 5 shows another silver eel, on the anterior part of whose head there were lesser tumors, while on the anterior dorsal portion of the body, that is on the pigmented skin, there were enormous confluent tumor formations a centimeter thick. Besides there was extensive tumor growth, for instance also on the tail, where the tumors were strongly pigmented. Finally I may mention that in four of the silver eels examined there was not the slightest trace of tumor formation on the head, but a large oblong or roundish, rather flat tumor on the side of the body, dorsal to the side line or

In summary it may thus as regards the location of the tumors be said that they seem to occur mainly on the head, especially on its anterior part, that they there attain very large dimensions, and that there often, besides, may be greater or lesser numbers of tumors scattered here and there on the body and fins, where they usually, however, are of comparatively smaller compass. Occurrence of the tumors on the body alone, without the head being affected, seems to be more uncommon. Besides, it has been noticeable that the growths for a much greater part have been located on the pigmented

portions of the skin, particularly dorsally to the side line, than on the lighter, ventral part of the body.

Most of the tumor-bearing eels sent us for examination were alive when received, and they were nearly all placed in aquaria or reservoirs so that we might study the further development of the growths. One of the aquaria was a salt water aquarium (0.5 per cent. NaCl), the others fresh water aquaria. The water was kept aerated partly by leading air through, partly by permanent afflux of running water; one large reservoir was placed outdoors, without any other aeration.

The viability of the eels was on the whole rather slight, and in that respect there was no difference between those placed in salt water and those placed in fresh water. Therefore, the observation time has been rather brief. Some kept alive, though, for two to four months, and could thus be observed for that length of time. But with a few exceptions the tumors did not undergo much change; in most of the cases neither increase in their size nor spread to fresh portions of the skin was observed. Only in one eel they gradually spread very considerably, so that they ended by involving large areas; and as this case is particularly interesting, it shall be described in some greater detail.

The animal was a silver eel, 68 cm. long, received Nov. 18th, 1947, from the Fisheries Control in Copenhagen (Section "Sound"). When received, it had large, dark red tumors both on the upper and lower jaw and on both pectoral fins. The largest tumor was the one on the lower jaw, which was entirely covered with a mass of tumor of the typical appearance, nearly as large as a walnut. On the anterior part of the upper jaw there was a very prominent tumor, which on both sides extended almost to the labial commissure and the anterior margin of the eye; but leaving both eyes free. On the upper, posterior part of the left pectoral fin there was a roundish, hazelnut-sized tumor, and at the base of the fin as well as on the dorsal edge of the latter several smaller, partly confluent ones. Also on the dorsal edge of the right pectoral fin there was a line of similar, lesser tumors. On the other parts of the skin there were none.

The eel was at once placed in a large open-air reservoir, together with some others with tumors. But on the bottom of the reservoir there were some leaves and other vegetal debris, a couple of boxes

Fig. 5. Silver eel with large, knobby tumors, especially on the dorsal aspect of the anterior part of the body and on the cauda fin. $^2/_5$ natural size. (M. Christiansen phot.).

with earth, etc., in which the animals could hide, and the eel in question was therefore not observed again until Jan. 19th, 1948, when it had come out again in a moribund state. It was then found that in the course of the two months the tumors had

Fig. 6. Silver eel with large, older tumors on the head and the left pectoral fin, and numerous younger, partly confluent, small, roundish tumors over the whole anterior part of the body; many of them—for instance on the part behind the pectoral fin—minute and just barely visible. $\frac{2}{3}$ natural size. (M. Christiansen phot.).

spread considerably, so that the whole anterior part of its body now, as Fig. 6 clearly shows, was actually covered with a dense crop of small tumors. On the picture one sees the original, very prominent tumor formations on the upper and lower jaw and on the left pectoral fin. Caudally to the tumor masses on the jaws there is a belt-shaped area, ventrally extending to the pectoral fin, dorsally somewhat shorter, where the skin—also of the non-pigmented ventral side—is thickly studded with minute tumors up to the size of a hemp seed. The left eye is now entirely covered by these young tumors, which form a complete layer over the whole area. The skin of the other part of the body seen in the picture is studded with masses of pale greyish nodules, some of them barely visible, others hardly as large as the head of a pin, but distinctly projecting. This is especially the case on the pigmented parts of the skin, while on the white, ventral side they are not nearly as numerous. Farther back, these small tumors become more scattered, also on the pigmented skin, and from about 10 cm. back of the pectoral fin they are not seen macroscopically. On the right side of the body, the crop of tumors is on the whole not quite as dense, in many places the young tumors are not situated as close to each other as on the left side. Thus the area about the right eye, and the eye itself, are not covered; there are only some small tumors here and there, a couple of them on the eye itself.

There has undoubtedly in this case been a rapid spread of multiple tumors over considerable parts of the skin; what the cause of it has been it is impossible to say, but from all we can see so far the phenomenon does not seem to be anything usual or common.

Histologic Examination.

Pieces of tumor from nearly all the eels received were removed for histologic study. The specimens were fixed in 10 per cent. formol, formol alcohol, Zenker's fluid with glacial acetic acid, or Heidenhain's "Susa"; then they were embedded in paraffin and stained, most of them with hemalum-eosin or with iron hematoxylin—picric acid—acid fuchsin (van Gieson).

Except for immaterial differences, the histologic structure of the examined tumors was very much the same. They were epithelial tumors of papillomatous structure. The stroma, except in the basal portion, was very scant, in the form of quite narrow, delicate streaks of connective tissue with sparse fibrils and with capillaries and fine vessels (Fig. 7). In some of the tumors, the stroma was more abundant, but still loose and well supplied with vessels; as a

Fig. 7. Section of tumor, showing the narrow streaks of connective tissue. Hemalum-eosin stain. × 45. (Photomicrograph by M. Christiansen).

rule, however, it was only in some few parts of the tumor that such a stronger development of the connective tissue stroma was found (Fig. 8).

Especially in the somewhat larger striae of connective tissue there are often deposits of black pigment in the form of larger or smaller clumps or streaks. The quantity in which this pigment is present varies considerably, though;—what also accords with the macroscopic appearance of the tumors, whose color, as we have seen, can vary from almost white to slate grey.

For the rest, the tumor tissue is composed of

Fig. 8. Section of tumor with more abundant connective tissue stroma, in which are seen small aggregates of pigment. Hemalum-eosin stain. × 45. (Photomicrograph by M. Christiansen).

proliferated epithelial cells. As Fig. 9 shows, the cells nearest the stroma are typical basal cells with elongated oval nuclei, arranged in rows in one or two layers. Inwards from these there are more roundish, irregularly disposed epithelial cells, whose nuclei are more roundish and less rich in chromatin. In slight enlargement, this layer, which may be of considerable thickness, therefore appears with a lighter color than the adjoining stratum of basal cells. Besides these types the tumors also contain the clavate cells and mucus-containing cells normally present in the skin of the eel; but the number in which these, which constitute a great part of the normal epidermis, occur in the different tumors varies considerably. In some there were only a very few, scattered here and there (Fig. 9), in others they were very dominant (Fig. 10), and between these extremes there were all sorts of transitions. They are always found in the central part of the epithelial formations, however.

To sum up, these formations thus consist of the following strata: 1) nearest the outside, i. e. lining

Fig. 9. Section of tumor. Delicate streaks of connective tissue with blood capillaries. Basal cells and more roundish tumor cells. Hemalum-eosin stain. × 230. (Photomicrograph by M. Christiansen).

the connective tissue stroma, the basal cells; 2) the more roundish epithelial cells; 3) farthest inward a

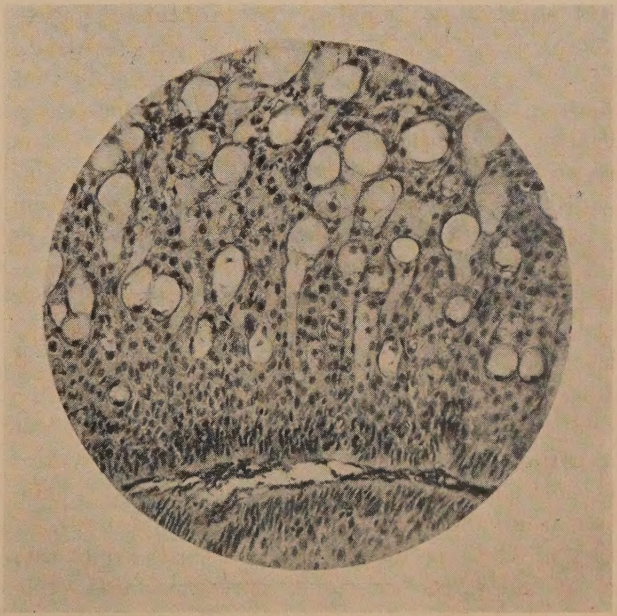


Fig. 10. Section of tumor, consisting largely of clavate cells and mucus-containing cells. Downward a streak of connective tissue with capillaries, limited on both sides by basal cells. Hemalum-eosin stain. × 190. (Photomicrograph by M. Christiansen).

greater or lesser number of mucous-containing cells and clavate cells.

Not even in the largest tumors were any necrotic processes observed either at gross inspection or at microscopic examination.

Attempts at Transmission.

The fact that the tumors do not seem to have been observed until in the last few years and that they during that time have become a not infrequent finding in eels in certain waters, naturally suggests the possibility that we may here have to do with an infectious disease, and that some contagium or pathogenic organism may perhaps be the cause of the tumors. We have therefore made various attempts to transmit the latter to sound eels. So far, our experiments in that direction have not succeeded, however; I shall therefore only mention them very briefly.

Simple contact experiments have been made in the way that eels with large tumor formations were placed together with sound eels—both yellow eels and silver eels—from waters in which the disease has not appeared, in small aquaria either with salt water (0.5 per cent. NaCl.) or fresh water; the contact being kept up as long as the eels with tumors remained alive, in some cases for two to three months.

In other experiments, inoculations from different tumors have been made on sound eels. The material for the inoculations was taken from the tumors as soon as the animal had been killed, and its further treatment and the subsequent inoculation were done as rapidly as possible. Before the material was taken, the surface of the tumor was sterilised by diathermy, whereupon it was taken from the deep part of the tumor under precautions for sterility. Owing to the lobulous structure of the tumors it is difficult, however, to ensure that the material is absolutely sterile. In some of the experiments we used as inoculation material lightly rubbed up tumor tissue emulsified in physiologic salt solution, with which the sound eel was then inoculated right under the skin of the extreme end of the snout and in the anterior chamber of the eye. In other series of experiments, the tumor was first strongly triturated with glass powder, then emulsified and left standing in refrigerator at $\div 5°$ C. for twenty-four hours, whereupon the cells were removed either by centrifugation or by filtration through a Seitz EK filter. The sound eels were then inoculated with this cell-free material intracutaneously (in the epidermis), or subcutaneously on a part of the skin close to one of the pectoral fins, right above the side line, where the epidermis had been scarified immediately before the inoculation. The eels thus inoculated were placed in aquaria and reservoirs. Some of them have remained alive for several months, one of them, inoculated with filtrate, now for over ten months. As already said, none of them has yet shown any signs of tumor formation.

Most of these experimental eels have been kept in reservoirs with running water, and thus under rather low temperature. In future transmission experiments we have planned to keep the eels at higher temperatures (about 15 to 17° C.), because it is possible that this may favor the development of the tumors, and thus also a possible transmission of them.

---

5. V. 1950.

found on or in the sea bottom in Danish waters. III. *C. G. Joh. Petersen:* The Work of the Biological Station from 1889—1914. With Illustrations and Tables. 88 pp. IV. *The same:* Appendix to Report XXI. With 2 Charts. 7 pp. On the distribution of the Animal Communities on the Sea Bottom. 1914.

XXIII. *C. G. Joh. Petersen:* On the Animal Communities of the Sea Bottom in the Skagerak, the Christiania Fjord and the Danish waters. With one Chart and four Tables. 29 pp. 1915.

XXIV. I. *C. G. Joh. Petersen:* On the Development of our Common Gobies (*Gobius*) from the Egg to the Adult Stages etc. With 1 Plate and 3 Figures in Text. II. *H. Blegvad:* On the Food of Fish in the Danish Waters within The Skaw. 72 pp. 1916.

XXV. *C. G. Joh. Petersen:* The Sea Bottom and its production of Fish-Food. A survey of the work done in connection with valuation of the Danish waters from 1883—1917. With 12 Plates and 1 Chart. 82 pp. 1918.

XXVI. I. *P. Boysen Jensen:* Valuation of the Limfjord. Studies on the Fish-Food in the Limfjord 1909—1917, its Quantity, Variation and Annual Production. With 7 Plates and 1 Chart. II. *C. G. Joh. Petersen:* Our Gobies *(Gobiidæ).* From the Egg to the Adult Stages. With 3 Plates. 65 pp. 1919.

XXVII. *C. G. Joh. Petersen:* On the stock of Plaice in relation to the intensive fishing of the present times in the Beltsea and other waters. With 2 Plates. 21 pp. 1920.

XXVIII. *H. Blegvad:* On the Biology of some Danish Gammarids and Mysids. *(Gammarus locusta, Mysis flexuosa, Mysis neglecta, Mysis inermis.)* With 5 Tables. 102 pp. 1922.

XXIX. *C. G. Joh. Petersen:* On the Stock of Plaice and the Plaice Fisheries in different Waters. With 2 Charts and 1 Appendix. 42 pp. 1922.

XXX. *R. Spärck:* Studies on the Biology of the Oyster *(Ostrea edulis)* in the Limfjord, with special Reference to the Influence of Temperature on the Sex Change. 80 pp. 1925.

XXXI. I. *C. G. Joh. Petersen:* The Influence of Fishing upon the Stock of Plaice in the Baltic during recent Years. — II. *R. Spärck:* Investigations on the Size of the Stock of Oysters in the Limfjord 1924. — III. *C. G. Joh. Petersen:* Report to the Ministry of Agriculture concerning increased Motor-Fishing in the Limfjord. — IV. *H. Blegvad:* Continued Studies on the Quantity of Fish-Food in the Sea Bottom. — V. *R. Spärck:* A Comparison between the Danish Oyster-Beds and those in Holland and the South-West of France. 63 pp. 1925.

XXXII. *H. Blegvad:* On the Renewal of the Stock of Plaice in the Baltic proper. 37 pp. 1927.

XXXIII. I. *A. C. Johansen:* On the Fluctuations in the Quantity of Young Fry among Plaice and certain other species of fish, and causes of the same. With 7 figures and 1 table. — II. *A. C. Johansen:* On a Spawning Place for winter spawning Herring in the northern part of the Belt Sea. With 2 figures. — III. *H. Blegvad:* On the Annual Fluctuations in the Age-composition of the stock of Plaice. With 2 figures. — IV. *R. Spärck:* Studies on the Biology of the Oyster *(Ostrea edulis)* II—IV. 65 pp. 1927.

XXXIV. I. *R. Spärck:* Studies on the Biology on the Oyster (*Ostrea edulis*) V. — II. *Aage J. C. Jensen:* Remarks on the Hydrography of the Limfjord. With 10 figures and 1 table. — III. *H. Blegvad:* Quantitative Investigations of Bottom Invertebrates in the Limfjord 1910—1927 with Special Reference to the Plaice-Food. With 2 figures and 2 tables. — IV. *A. C. Johansen:* The Extent of the Annual Transplantations of Plaice in the Limfjord and the Yield resulting therefrom. With 11 figures and 12 tables. — V. *Aage J. C. Jensen:* The Relation between the Size of the Plaice Stock and the Quantity of "First Class Plaice-Food" in certain Parts of the Limfjord. With 3 figures and 2 tables. — VI. *Erik M. Poulsen:* The Haddock in the Belt Sea and the Western Baltic during the Years 1926—28. With 4 figures and 8 tables. 125 pp. 1928.

XXXV. I. *A. C. Johansen:* The Aalborg Herring and its Importance to the Danish Herring Fishery from the XVIth Century until the Present Day. With 2 figures and 5 tables. II. *H. Blegvad:* Mortality among Animals of the Littoral Region in Ice Winters. With 3 figures. III. *A. C. Johansen:* Mortality among Porpoises, Fish, and the Larger Crustaceans in the Waters around Denmark in Severe Winters. With 2 figures. IV. *R. Spärck:* Studies on the Biology of the Oyster (*Ostrea edulis*) VI—VIII. 109 pp. 1929.

XXXVI. I. *H. Blegvad:* Quantitative Investigations of Bottom Invertebrates in the Kattegat with Special Reference to the Plaice Food. With 1 chart and (8+6) tables. *A. C. Johansen:* The Sprat (*Clupea sprattus* L.) in the Limfjord. With 4 figures and 1 table. III. *Aage*

J. C. Jensen: On the Influence of hydrographical Factors upon the Yield of the Mackerel Fishery in the Sound. With 2 figures and 17 tables. IV. *H. Blegvad:* Aerial Spotting of Fish Shoals. Danish Experiments in 1930. With a chart. 96 pp. 1930.

XXXVII. I. *H. Blegvad:* Investigations of the Bottom Fauna at Outfalls of Drains in the Sound. With 4 figures and 2 tables. II. *Aage J. C. Jensen:* Hydrographical Investigations of the Pollution of the Sound at Copenhagen. With 4 figures and 1 table. III. *Erik M. Poulsen:* Analyses of Stock and Race of the Baltic Plaice during recent Years. With 3 figures and 3 tables. IV. *R. Spärck:* Studies on the Biology of the Oyster in the Limfjord. IX. V. *R. Spärck:* On the Capacity of Migration of Adult Individuals of *Asterias rubens.* VI. *R. Spärck & E. Lange:* Preliminary Investigation on the Content of Bacteria in the Limfjords Oyster. VII. *H. Blegvad.* On Aerial Spotting of Fish Shoals. II. Danish Experiment in 1931. With 1 chart. VIII. *H. Blegvad:* Preliminary Report on the Danish Experimental Transplantations of Plaice from the North Sea to the Belt Sea during the Years 1928– 31. With 4 figures. 94 pp. 1932.

XXXVIII. I. *H. Blegvad:* S/S "Biologen". With 14 figures. II. *Erik M. Poulsen:* Biology of the Dab in Danish Waters. With 7 figures and 23 tables. III. *R. Spärck:* Investigations on the Biology of the Oyster X. 32 pp. 1933.

XXXIX. I. *H. Blegvad:* An Epidemic Disease of the Eel-Grass *(Zostera marina* L.*)* With 1 Chart. II. *H. Blegvad:* Transplantations of Plaice from the North Sea to the Belt Sea. 1928—1933. The Scientific Survey. With 43 figures and 23 tables. 84 pp. 1934 (printed 1935).

XL. I. *Henning E. Petersen:* Preliminary Report on the Disease of the Eelgrass (*Zostera marina* L.) With 4 figures. II. *Erik M. Poulsen:* Recent Investigations into the Stock of Salmon and Sea Trout in the River Gudenaa. With 8 figures and 9 tables. III. *R. Spärck:* Investigations on the biology of the oyster XI. On the size and age-composition of the stock of native oyster in 1935. IV. *R. Spärck:* On the occurrence of *Crepidula fornicata* (L.) in the Limfjord. 44 pp. 1935.

XLI. I. *Knud Larsen:* The Distribution of the Invertebrates in the Dybsø Fjord, Their Biology and Their Importance as Fish Food. With 9 figures and 3 tables. II. *Søren Lund:* On the Production of Matter and the Growth in some Benthic Plants. With 8 figures and 6 tables. 52 pg. 1936.

XLII. I. *C. V. Otterstrøm:* On Methodical Liberations of Salmon and Trout Fry in Watercourses with Special Reference to the Gudenaa Area. With 21 figures. II. *Erik M. Poulsen:* The Dab in the Danish Waters, Investigations on Stock and Growth. With 12 figures and 6 tables. III. *Aage J. C. Jensen:* The Races of Dab in the Danish Waters. With 4 figures and 2 tables. 63 pg. 1937 (printed 1938).

XLIII. *Erik M. Poulsen:* On the Migrations and the Racial Character of the Plaice. With 10 figures and 8 tables. 80 pg. 1938 (printed 1939).

XLIV. *C.V. Otterstrøm* and *E. Steemann Nielsen:* Two Cases of Extensive Mortality in Fishes Caused by the Flagellate *Prymnesium parvum,* Carter. With 9 figures. 24 pg. 1939 (printed 1940).

XLV. *H. Blegvad:* The Danish Biological Station through 50 Years 1889—1939. With 64 figures. 69 pg. 1940 (printed 1944).

XLVI. *Erik M. Poulsen:* On Fluctuations in the Size of the Stock of Cod in the Waters within the Skaw during Recent Years. With 13 figures. 36 pg. 1941 (printed 1945).

XLVII. I. *Søren Lund:* On Colpomenia peregrina Sauv. and its Occurrence in Danish Waters. With 5 figures. II. *Knud Larsen:* The Liberation af Salmon and Trout Fry in Denmark. With 3 figures and 1 table. III. *C.V. Otterstrøm:* Turbines and Descending Salmon and Trout Smolts (and Eels): With 10 figures. IV. *Frode Bramsnæs, Mogens Jul* and *C.V. Otterstrøm:* Barriers against Fishes by Means of Veils of Air. With 3 figures. 46 pg. 1942. (printed 1945).

XLVIII. I. *H. Blegvad:* On the Waste Fish Fishery. With 2 figures and 8 tables. II. *Erik M. Poulsen:* Investigations on the Danish Fishery for and the Biology of the Norway Lobster and the Deep-sea Prawn. With 12 figures and 7 tables. III. *C. V. Otterstrøm* and *Knud Larsen:* Extensive Mortality in Trout Caused by the Infusorian Stentor polymorphus Ehrenb. With 2 figures. 57 pg. 1943—45 (printed 1946).

XLIX. I. *Erik M. Poulsen:* Seine Fishery for Small Herring and Sprat in the Limfjord. With 6 figures and 10 tables. II. *Knud Larsen:* First Report on the Effect of the Liberation of Salmon Fry in the Gudenaa 1946—47. With 8 figures. 37 pg. 1946 (printed 1949).

Printed by Bianco Luno, Copenhagen.